

EFFECTS OF INTRACEREBRAL PROSTAGLANDIN E₂ UPON
SEXUAL RECEPTIVITY, OPEN FIELD ACTIVITY BEHAVIOR
AND BODY TEMPERATURE IN OVARECTOMIZED FEMALE RATS

By

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Dedicated to the memory of those magnificent vermin
who so gallantly gave up their brains so that the
author could increase the capacity of his own.

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In nearly all endeavors, the finished product represents but a fragment of the total effort expended to achieve the goal. This dissertation is not an exception. Although only one name appears as the author of this manuscript, other names deserve recognition for contributing in so many ways toward its completion. Hazel, my loving wife, who kept me from developing apoplexy when certain experiments went awry. Dr. William Luttge, a friend and my advisor, whose guidance from the time that this project was conceived kept the study within the realm of science. The members of my committee, Drs. Bernstein, Dewsbury, King, Luttge, Kalra and Zornetzer, for their constructive critique of the original proposal. Richard Berry for assisting with the behavioral tests during Experiment 1. Max Hicks and Craig Tepper for technical assistance throughout the study. Special thanks are due Barbara McGuire for her invaluable assistance and advice during the histologic preparation of the brains. Again, my wife Hazel for her assistance in preparing the tissue for histology. Pat Dreyer for typing this disquisition and for making it appear more splendid than it probably actually is. Finally, the U.S. taxpayer whose blood supported me during the past 4 years and the personnel of animal care facilities for keeping my rats in their correct cages.

TABLE OF CONTENTS

	<u>Page</u>
ACKNOWLEDGMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT	viii
INTRODUCTION	1
EXPERIMENT 1	
EFFECTS OF INTRAVENTRICULAR PROSTAGLANDIN E ₂ ON	
SEXUAL RECEPTIVITY	30
Methods	31
Results	35
EXPERIMENT 2	
INDUCTION OF SEXUAL RECEPTIVITY WITH PGE ₂	
IMPLANTS IN SPECIFIC SUB-CORTICAL REGIONS	44
Methods	44
Results	50
EXPERIMENT 3	
EFFECTS OF SYSTEMIC AND INTRACEREBRAL INDOMETHACIN	
ON ESTRADIOL AND PROGESTERONE INDUCED SEXUAL	
RECEPTIVITY	61
Part A Methods	62
Part A Results	65
Part B Methods	70
Part B Results	70
Part C Methods	72
Part C Results	74
EXPERIMENT 4	
EFFECTS OF ADRENALECTOMY ON PGE ₂ INDUCED SEXUAL	
RECEPTIVITY	78
Methods	78
Results	80
DISCUSSION	86
Conclusion	95
APPENDIX	99

	<u>Page</u>
REFERENCES	109
BIOGRAPHICAL SKETCH	122

LIST OF TABLES

	<u>Page</u>
TABLE 1 Injection And Testing Paradigm For Intra- ventricular PGE ₂	33
TABLE 2 Lordosis Quotients Over Time Following Intraventricular PGE ₂ or Cholesterol . . .	39
TABLE 3 PGE ₂ And Cholesterol Implantation Schedule	46
TABLE 4 Injection And Testing Paradigm For Intra- cerebral PGE ₂	49
TABLE 5 Individual Lordosis Quotient Scores Follow- ing Intracerebral PGE ₂ And Cholesterol (Chol)	51
TABLE 6 Individual Activity Scores Following Intra- cerebral PGE ₂ or Cholesterol (Chol) . . .	55
TABLE 7 Individual Rectal Body Temperature Measures Before And After Intracerebral PGE ₂	57
TABLE 8 Individual Rectal Body Temperature Measures Before And After Intracerebral Cholesterol	59
TABLE 9 Injection And Testing Paradigm For Indo- methacin Experiments	63
TABLE 10 Mean ± SEM Rectal Body Temperature (°C) Following Systemic And Intracerebral Indomethacin	73
TABLE 11 Injection And Testing Paradigm Following Adrenalectomy	79

LIST OF FIGURES

	<u>Page</u>
FIGURE 1 Highest receptivity score exhibited on a given test day following intraventricular implants of PGE ₂ or cholesterol	37
FIGURE 2 Rectal body temperatures of animals receiving implants of either PGE ₂ , cholesterol or an empty cannula	43
FIGURE 3 Receptivity scores of rats receiving 5, 7.5 or 15 mg of indomethacin injected sc or 30 µg of crystalline indomethacin implanted into the anterior hypothalamus	67
FIGURE 4 Open field activity scores of rats receiving 5, 7.5 or 15 mg of indomethacin implanted into the anterior hypothalamus	69
FIGURE 5 Receptivity scores of ovariectomized and adrenalectomized rats at 7 weekly intervals with sc estrogen and PGE ₂ , progesterone and/or corticosterone	82
FIGURE 6 Open field activity scores of ovariectomized and adrenalectomized rats tested at 7 weekly intervals with sc estrogen and PGE ₂ , progesterone and/or corticosterone	85
APPENDIX FIGURE 1 Sites of PGE ₂ implants in animals used in Experiment 2	100

Abstract of Dissertation Presented to the Graduate Council
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EFFECTS OF INTRACEREBRAL PROSTAGLANDIN E_2 UPON
SEXUAL RECEPTIVITY, OPEN FIELD ACTIVITY BEHAVIOR
AND BODY TEMPERATURE IN OVARIETOMIZED FEMALE RATS

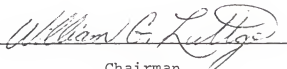
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To determine whether Prostaglandin E_2 (PGE_2) might be part of the neural mechanism mediating sexual receptivity, the compound was implanted into discrete sub-cortical brain regions of estrogen primed, ovariectomized rats. Highest levels of receptivity were observed following implants in the anterior hypothalamus and preoptic area. Sexual behavior was also observed when PGE_2 was implanted into the third ventricle, medial basal hypothalamus, and anterior mesencephalon. Open field activity levels were the same as control values following PGE_2 implants into all of the brain sites examined. Adrenalectomy significantly lowered the receptivity scores. However, the adrenalectomized animals were also extremely lethargic while the PGE_2 implants were in place. This lethargy could not be reversed with progesterone or corticosterone therapy. Rectal body temperature was elevated significantly following PGE_2 implants into the third ventricle and hypothalamic sites. Systemic and intracerebral indomethacin failed to inhibit estrogen and progesterone induced sexual receptivity

suggesting that although PGE_2 can facilitate the display of lordosis, it probably is not part of the mechanism by which estrogen and progesterone exert their effects on behavior.


Chairman

INTRODUCTION

Several mechanisms have been suggested to explain the effects of steroid elicitation of sexual receptivity in female rodents. Estrogen and progesterone could act directly upon neurons that mediate sexual behavior or they might cause the release or inhibition of neurotransmitters which in turn act upon behaviorally sensitive areas. Evidence exists that implicates both serotonin and catecholamines in playing such a role. Another possibility is that estrogen induces the release of luteinizing hormone releasing factor (LRF) which acts either at the cells directly or acts via a neurotransmitter system. This introduction will review the effects of estrogen at estrogen sensitive receptor cells, a proposed mechanism for the control of female sexual behavior involving progesterone and serotonin, the effects of catecholamines, the possibility that LRF might play a role, and ways by which prostaglandin E_2 (PGE_2) might interact with LRF to also exert an effect upon sexual receptivity.

A considerable amount of research has been conducted in order to elucidate the mechanism by which estrogen, as well as other steroid hormones, activate receptor cells.

Basically, six steps are involved between the arrival of the hormone at the target cell and the functional response of the tissue. These steps have been reviewed in detail by O'Malley and Means (1974) and can be briefly summarized as follows. Initially, the hormone is taken up and bound in the target cell by a cytoplasmic receptor. Most of the evidence suggests that this receptor is a protein. A complex of the receptor and hormone is then transported to the cell nucleus. Acceptor sites on the genome then bind this protein-steroid complex prior to activation of the transcription apparatus. New RNA is induced by the hormone and this in turn is transported to the cytoplasm where new proteins are synthesized. The final step consists of the functional response that is typical of the particular target issue involved. Most of the research leading to the development of this model was done using the chick oviduct and rat uterine tissue (O'Malley and Means, 1974). But evidence exists that stimulation of protein synthesis might also be involved in the activation of estrogen sensitive brain cells that mediate female sexual behavior.

Quadagno, Shryne, and Gorski (1971) were one of the first groups to demonstrate that the RNA synthesis inhibitor, actinomycin-D, can inhibit steroid induced sexual behavior in female rats. The inhibition was dependent upon the priming dose of estrogen as well as the time of administration following the estrogen. Bilateral implantation

or subcutaneous administration of actinomycin-D into the hypothalamus significantly inhibited lordotic behavior when given 12 hours after 3 μ g of estrogen. The same dose of actinomycin-D did not block the behavioral effects of 15 μ g of estrogen or 3 μ g of estrogen when the blocker was given 32 hours after the steroid. Similar findings were reported by Terkel, Shryne and Gorski (1973) following infusion of actinomycin-D into the third ventricle or pre-optic area. In addition, the inhibitory effects of the drug were found to be reversible. Since actinomycin-D blocks DNA dependent RNA synthesis, these results supported the hypothesis that RNA synthesis is necessary for estrogen induced sexual behavior. Because the above studies involved infusion of the drug into the ventricles or else into hypothalamic regions where exposure to the ventricles was almost certain, the precise site of action of actinomycin-D could not be ascertained. Consequently, another group attempted to localize more precisely the brain sites where blocking protein synthesis interferes with female sexual receptivity (Whalen, Gorzalka, DeBold, Quadagno, Ho and Hough, 1974). Although the results were inconclusive, they did suggest that the preoptic area was the most effective site. Receptivity was inhibited with implants of actinomycin-D in this area and when the cannulas did not pierce the ventricles. However, caudate implants were ineffective even if the cannulas penetrated the lateral ventricle. The same authors also reported that implants

located in the third ventricle were very effective in inhibiting receptivity. Thus, the precise neural substrate remains a mystery. Cells in the preoptic area were responsive to the protein synthesis inhibitor, but whether the ventricular system carried the drug to other effective sites or simply provided quick access to the preoptic region cannot be answered based upon the present literature.

Correlated with the decline in sexual receptivity are structural changes in nucleolar morphology in the preoptic cells (Ho, Quadagno, Cooke and Gorski, 1973/1974). Following the implantation of actinomycin-D, the fibrillar and granular components of the nucleolus became segregated into separate areas. This "nucleolar segregation" is correlated with the inhibition of RNA synthesis (see Hough, Ho, Cooke and Quadagno, 1974). As in the case of sex behavior, the drug was found to have a reversible effect on the nucleolar morphology. The results of Hough et al. (1974) also indicated a correlation between normal levels of sexual receptivity and normal nucleolar morphology.

Other protein synthesis inhibitors such as cycloheximide exert similar inhibitory effects upon estrogen mediated behaviors including copulatory behavior, wheel running activity and sexual motivation (Meyerson, 1973). Hough et al. (1974) discussed the implications of their findings in the perspective of the hypothesis that the preoptic area exerts an inhibitory influence over receptivity,

possibly via other brain regions. One mechanism that was considered was estrogen induced synthesis of an inhibitory protein that results in disinhibition of this influence and ultimately facilitates sexual behavior. An estrogen induced protein has been demonstrated to exist in the uterus (O'Malley and Means, 1974). If actinomycin-D or cycloheximide prevent the synthesis of such an estrogen induced protein, then it would explain why these drugs were able to interfere with steroid induced sexual receptivity.

In addition to direct stimulation of proteins, other theories implicating neurotransmitters in steroid-stimulated receptivity have also been proposed. One such theory implicates serotonergic cells of the raphe nuclei, especially in the mediation of progesterone's effects (Meyerson, 1964a,b, 1966, 1968; Zemlan, Ward, Crowley and Margules, 1973; Kow, Malsbury and Pfaff, 1974). Briefly, the theory states that progesterone's facilitatory effects are due to inhibition of activity in a raphe-septal loop that includes serotonergic fibers and which inhibits lordosis (Kow et al., 1974). This theory has gained support from neuroanatomical as well as lesion and drug studies.

Conrad, Leonard and Pfaff (1974) have described fiber connections of the medial and dorsal raphe nuclei in the rat. They combined autoradiographic and degeneration techniques and found that most of the fibers project ventrally and then pass through the ventral tegmentum and into the medial forebrain bundle. From the medial forebrain bundle,

fibers were found to pass into the hypothalamus, preoptic area, amygdala and olfactory tubercle. However most of the medial forebrain bundle fibers were found to continue rostrally joining the diagonal band of Broca to reach the septal nuclei. Other projections include one to the habenular nucleus through the fasciculus retroflexus. It had previously been shown by Smaha and Kaelbar (1973) that fibers project forward from the habenular nucleus into the septum. Not only is there input from the raphe nuclei to the septum, but septal output also reaches the raphe. Some septal fibers project into the medial forebrain bundle. Others pass through the stria medullaris to the medial habenular from where they project to the medial raphe nucleus (Raisman, 1966; Cragg, 1961; Conrad and Pfaff, 1974). Several lines of evidence have implicated this raphe-septal loop, which may involve serotonin as well as other neurotransmitters, with behavior.

Septal lesions can increase sensitivity to somatic stimuli (Carey, 1972). This is relevant since stimulation from the male during copulation consists in part of flank palpation which is a form of somatic stimulation. Lesions in the midbrain raphe nuclei also increase sensitivity to somatic stimuli (Lints and Harvey, 1969). These effects might be mediated by serotonin since the injection of parachlorophenylalanine (PCPA), which decreases brain levels of serotonin, also results in heightened sensitivity to external stimuli in rats (Tenen, 1967). It has also been

shown that in females primed with estrogen, septal destruction enhances lordosis (Komisaruk, Larsson and Cooper, 1972; Nance et al., 1974). Conversely, electrical stimulation of the septum inhibits lordosis in estrogen and progesterone primed rats (Malsbury and Pfaff, 1973). Electrical stimulation of the septum also decreases responsiveness to external stimulation (Kostowski, Giacalone, Garattini and Valzelli, 1969; Gumalka, Samanin, Valzelli and Consolo, 1971; Liebeskind, Guilbaud, Besson and Oliveras, 1973). Finally, Meyerson (1964b, 1966, 1968) has demonstrated that increasing brain levels of serotonin by injecting the precursor, 5-hydroxytryptophan, inhibits the display of lordotic responding in female rats. Thus, there is evidence that septal stimulation and serotonin elevation decreases lordotic responding while septal lesions and serotonin depletion increases lordotic responding. That progesterone might be directly involved in this serotonin system has been suggested by several reports that the midbrain region of the rat brain, which includes serotonergic cell bodies, accumulates more radioactive progesterone than any other brain region (Whalen and Luttge, 1971a,b; Luttge and Wallis, 1973; Luttge, Chronister and Hall, 1973; Luttge, Wallis and Hall, 1974).

On the basis of these data, it has been hypothesized that serotonergic fibers from the raphe complex inhibit responses to somatosensory input including inhibition of lordosis (Kow et al., 1974). Responses to somatosensory input as

well as lordotic responding, are facilitated by disrupting function within this anatomical loop. Because progesterone has been shown to decrease responses to sensory stimuli by hypothalamic as well as by other neurons (Barraclough and Cross, 1963; Komisaruk, McDonald, Whitmoyer and Sawyer, 1967; Ramirez, Komisaruk, Whitmoyer and Sawyer, 1967), it has been suggested that progesterone might also inhibit neuronal activity within the raphe-septal loop. If such inhibition does occur, then based upon the previously described evidence, it should result in a facilitation of lordotic responding. If progesterone is indeed causing inhibition, then this steroid should facilitate receptivity when implanted into the serotonergic cells of the raphe complex. Although this prediction has received some support, there is evidence that the system is more complex than simply inhibition of the serotonergic system. Ross, Claybaugh, Clemens and Gorski (1971) did report an increase in lordotic responding with progesterone implants in the ventral mesencephalon of estrogen primed, ovariectomized rats. But Powers (1972) found no facilitation with progesterone in this general area although it is important to note that none of his implants were located in the ventral mesencephalon. Most of his implants were located dorsal to this area. Instead, Powers (1972) reported facilitation with progesterone implants in the medial basal hypothalamus. A more recent study has revealed that only when the progesterone implants are located in the interpeduncular-ventral

tegmental area is full receptivity observed (Luttge, submitted for publication). Other studies in different species have reported inhibition of receptivity with mesencephalic implants of progesterone. Morin and Feder (1974) reported inhibition using the guinea pig and Hall and Luttge (1974) found inhibition in the mouse.

These findings suggest that progesterone does more than to simply inhibit neuronal activity within the serotonergic system. For example, different metabolites of progesterone could subserve different functions in the mediation of receptive behavior as has been demonstrated by Czaja, Goldfoot and Karavolas (1974) and Luttge and Hall (1976). There is additional evidence that different neural mechanisms might be involved depending upon whether receptivity is induced by treatment with chronic estradiol or estradiol plus progesterone. Espino, Sano and Wade (1974) reported that the serotonin agonist α -methyltryptamine blocked receptivity, but only when the ovariectomized rats were primed with estradiol plus progesterone. The drug had no effect on receptivity induced by high doses of estradiol alone. It has also been found that habenular lesions block receptivity in females treated with estradiol plus progesterone but not in females treated with large amounts of estradiol. An alternative or second independent mechanism explaining these differential effects might involve other neurotransmitters.

Monoamine involvement in the control of female sexual

behavior has been suggested by a number of studies that showed that ovarian steroids are able to affect cerebral monoamine function. During the rat estrous cycle, there are fluctuations in hypothalamic monoamine oxidase activity (Zolovic, Pearse, Boehlke and Eleftheriou, 1966) and norepinephrine levels (Stefano and Donoso, 1967). These fluctuations are manifested by increases in monoamine oxidase activity and decreases in norepinephrine levels during estrous. Intact female rats receiving exogenous estrogen have reduced norepinephrine levels in the anterior hypothalamus (Donoso and Cukier, 1968) while ovariectomized rats have increased norepinephrine and decreased dopamine levels. These latter effects are reversible with large doses of estrogen and progesterone (Donoso and Stefano, 1967). More direct evidence for the involvement of catecholamines in the mediation of female sexual behavior was provided by Ahlenius, Engel, Eriksson and Sodersten (1972) and Ahlenius, Engel, Eriksson, Modish and Sodersten (1972). Tetrabenezene, a potent monoamine depleter, was administered to ovariectomized female rats in the first study for the purpose of monitoring brain monoamine levels and sexual receptivity. Following the administration of this drug, there was a decrease in brain dopamine and a concurrent increase in the number of lordotic responses. When a higher dose of tetrabenezene was administered, significant decreases of norepinephrine and serotonin were observed. As with dopamine, the decreased levels of the latter two

neurotransmitters were correlated with increased receptivity. The second study by this same group of investigators examined the effects of α -methylparatyrosine (α -MT) and PCPA on the same parameters. Both treatments were found to be correlated with a transient decrease in norepinephrine and dopamine that was correlated in time with the facilitation of lordotic responding. It was especially interesting that although PCPA resulted in a gradual decline in brain serotonin, there was no increase in lordotic behavior when the depletion was most pronounced. Lordotic behavior corresponded with the restoration of control levels of brain catecholamines suggesting a minimal role for serotonin in contradiction to the evidence discussed earlier. Another study directly implicating brain catecholamines in the mediation of sexual behavior was conducted by Herndon and Neill (1973) using brain lesioned animals. Although certain brain lesions enhance receptivity by apparently disrupting inhibitory mechanisms, other areas, when damaged have been found to result in inhibition of receptivity. One such area is the anterior hypothalamus. Bilateral lesions in this region have been found to disrupt receptivity in estrogen and progesterone primed ovariectomized rats (Herndon and Neill, 1973). But when the animals were given 1 mg/kg body weight of d-amphetamine sulfate following these lesions, their receptivity scores were restored to the levels exhibited by nonlesioned control animals. Since d-amphetamine functions primarily to facilitate

catecholaminergic neurotransmission, the drug's effect was thought to be related to catecholamine release although serotonin may have been released as well (Reid, 1970). The results of the Herndon and Neill (1973) study were interpreted as being suggestive of an excitatory role for catecholamines in contradiction to the inhibitory role assigned to at least dopamine by Ahlenius et al. (1972, 1972).

Not only is there confusion in regards to neurotransmitter mediation of sexual behavior because of the nonspecificity of many of the drugs used, but it has been suggested that some of these effects might be mediated by adrenal progesterone. One such study (Paris, Resko and Goy, 1971) found that estrogen primed ovariectomized rats displayed lordotic responses with concurrent increases in plasma progesterone levels following the administration of reserpine. This effect was blocked, however, if the animals were adrenalectomized, hypophysectomized or if they were given dexamethasone which inhibits ACTH secretion. Lordosis was also seen if estrogen primed animals received metopirone, a corticotropin activator. Consequently, it was suggested that although reserpine influences sexual receptivity, it is not via its action on neural tissue regulating behavior but by stimulation of ACTH and hence adrenal progesterone. Similar adrenal effects have been reported by Eriksson and Sodersten (1973) and Gorzalka and Whalen (1974). Others, however, have used adrenalectomized animals as controls and found no role for adrenal mediation of these effects

(Zemlan et al., 1973; Meyerson, 1964b).

In an effort to resolve some of these confusing issues, Everitt, Fuxe and Hokfelt (1974) combined various drug treatments with the appropriate control procedures in order to elucidate the role played by specific neurotransmitters and adrenal progesterone. Ovariectomized or ovariectomized and adrenalectomized rats were used and their behavior was quantified and qualified following the various treatment paradigms. The results showed that pimozide, a potent dopamine receptor blocker, facilitated all measures of female sexual behavior. These effects were potentiated by d-amphetamine. Although d-amphetamine stimulates the release of both dopamine and norepinephrine, the dopamine was ineffective in the presence of pimozide suggesting that the facilitation was due to norepinephrine. The involvement of norepinephrine was further suggested when chlorpromazine, which blocks both norepinephrine and dopamine receptors, was ineffective in stimulating lordotic behavior with d-amphetamine. PCPA also was found to result in a significant increase of all of the components of receptivity. This effect was found to continue after catecholamine levels were supposed to have been restored to normal, contradicting the previously discussed conclusions of Ahlenius et al. (1972, 1972). The effects of PCPA and pimozide were unaffected by adrenalectomy except on the measure of lordosis intensity which was found to be consistently decreased. It was suggested that this finding might

explain the results of those reporting an adrenal effect, especially if only full lordotic postures were considered as indices of receptivity. Opposite effects were observed after the administration of drugs that enhanced the activity of dopamine and serotonin. ET 495, a dopamine stimulating agent, was found to decrease significantly estrogen and progesterone induced receptivity. As a consequence of this study, the authors concluded that dopamine and serotonin subserve inhibitory roles in mediating sexual receptivity while norepinephrine might have a slight excitatory function.

It is important that none of the results discussed thus far exclude any one neurotransmitter system from playing some role in mediating receptivity. It is possible that serotonin and catecholamines are each influencing different components of receptivity or that neurotransmitters interact to influence the behavior. There is biochemical evidence for such an interaction. Blondaux, Juge, Sordet, Chouvet, Jouvét and Pujol (1973) found that intracisternal administration of 6-hydroxydopamine, a drug used to destroy catecholaminergic neurons in rats, resulted in a substantial increase in both serotonin synthesis as well as the 5-HIAA concentration in the telencephalon. It has also been shown that in cats, inhibition of catecholamine synthesis using α -MT causes a marked increase in serotonin synthesis (Jouvét, 1972). In order to explore possible interactions between the raphe neurons and those of the locus coeruleus,

Kostowski, Samanin, Bareggi, Marc, Garattin and Valzelli (1974) lesioned one or the other of these two nuclei groups and then measured brain levels of serotonin and its metabolite 5-HIAA and norepinephrine and its metabolite, 3-methoxy-4-hydroxyphenylglycol sulfate (MOPEG-SO₄). It was found that after bilateral lesions of the locus coeruleus, a significant increase of forebrain 5-HIAA concentration resulted four days after surgery. Serotonin levels remained unchanged while hippocampal and cortical levels of norepinephrine were slightly reduced. The slight increase in forebrain 5-HIAA was not significant ten days after the operation. Lesions of the ventral raphe nucleus produced no change in the hippocampal and cortical levels of norepinephrine, however, after four days, MOPEG-SO₄ levels were significantly increased. This latter increase in the norepinephrine metabolite following destruction of the serotonergic cells suggests that these neurons in some way influence the metabolism of norepinephrine or that norepinephrine also originates in the vicinity of the serotonergic cells (Lindvall and Bjorklund, 1975). Further support for the involvement of monoamines in the mediation of receptive behavior is to be found in the results of studies showing both catecholaminergic and serotonergic fibers in areas of the hypothalamus implicated in the mediation of sexual receptivity (Ungerstedt, 1971; Swanson and Hartman, 1974; Saavedra, Palkovits, Brownstein and Axelrod, 1974; Bjorklund, Lindvall and Nobin, 1975). Direct effects of the gonadal

hormones on the uptake of radiolabelled monoamines in the rat brain are also known. Wirz-Justice, Hackman and Lichsteiner (1974) examined the uptake of ^3H -dopamine and ^3H -serotonin following estrogen alone, progesterone alone or estrogen followed 48 hours later by progesterone. Estrogen alone decreased dopamine uptake in the thalamus while estrogen plus progesterone decreased dopamine uptake in the cortex, preoptic area and septal region. Progesterone, however, was found to increase serotonin uptake in the preoptic and septal regions. Thus, estrogen, either alone or in combination with progesterone, only influenced dopamine uptake while progesterone alone only influenced the uptake of labelled serotonin. Similar findings had been reported previously when Janowsky and Davis (1970) demonstrated that estrogen and progesterone significantly inhibited the uptake of tritiated norepinephrine by isolated rat brain synaptosomes. The possibility that estrogen and progesterone influence sexual behavior via different neurotransmitter systems, supporting the hypothesis that estrogen mediated behavior involves a mechanism separate from that mediated by estrogen plus progesterone, cannot be ruled out. It is also possible that the catecholamines are able to influence receptivity indirectly via LRF since both norepinephrine and dopamine have been shown to stimulate LH release and presumably via the releasing factor (McCann, 1974; Kalra and McCann, 1973).

The cholinergic neurotransmitter system has also been

implicated in the control of sexual receptivity in female rats. One of the first studies to suggest a role for this system was conducted by Lindstrom and Meyerson (1966). They reported that pilocarpine and arecoline decreased receptive behavior and that atropine, a cholinergic receptor blocker, antagonized this inhibition. In a subsequent study, the same authors (Lindstrom and Meyerson, 1970) reported that atropine by itself had no effect on estrogen and progesterone induced receptivity in ovariectomized rats but that it exerted an inhibitory effect when preceded by the monoamine oxidase inhibitor pargyline or nialamide which alone also had no effect. An inhibitory effect was also reported when the order of the atropine and pargyline injections was reversed. Contradicting these studies was one conducted by Singer (1968) in which he found an inhibitory effect with atropine alone although a larger dose was used than in the Lindstrom and Meyerson (1966, 1970) studies. Hardy and Warburton (1969) confused the issue even further when they reported no changes in sexual receptivity after acetylcholine levels were altered using di-isopropyl-fluorophosphate (DFP). This drug lowers brain cholinesterase activity and results in an effective increase in acetylcholine levels. Despite induced fluctuations in acetylcholine levels, ovariectomized rats treated with either estrogen alone or estrogen plus progesterone exhibited no changes in sexual receptivity. However, measures of male rejection were found to increase significantly after four days of the drug injection.

There is equally inconclusive evidence that acetylcholine might be involved in gonadotropin release and hence, might exert an influence via the LRF mechanism. Cholinergic synapses have been found in the hypothalamus (McCann, 1974) and it has been shown that atropine can block ovulation (Markee, Everitt and Sawyer, 1952). A more recent study has verified this observation by demonstrating that administration of atropine either subcutaneously or via the third ventricle can block the proestrus release of gonadotropins (Libertun and McCann, 1973). But since carbochol, which mimics the action of acetylcholine, has not been found to cause an immediate increase in gonadotropin release (McCann, 1974) further investigation is required before assigning a role to acetylcholine in the control of LH release.

During the past two years, evidence has been reported to suggest that LRF might also play a role in controlling sexual receptivity. Moss and McCann (1973), Moss (1974) and Moss, McCann and Dudley (1975) have described a series of experiments that provide convincing data that LRF is able to induce receptivity. Six groups of ovariectomized rats were pretreated with estrone followed by progesterone, LRF, LH, FSH or TRF. Only those animals treated with either progesterone or LRF displayed sexual receptivity. Animals receiving LH, FSH or TRF showed little or no lordotic responding. Furthermore, receptive animals receiving LRF or progesterone were observed to display the sex soliciting behaviors, hopping and darting. The effectiveness of the LRF was dependent

upon both the dose and time after injection of estrogen that it was administered. A steady rise in the lordosis quotient from approximately 0.08 to 0.58 over a dose range of 50 to 500 ng of LRF was found. Higher doses did not increase the scores. Although the greatest amount of responding occurred eight hours after LRF, scores of 60 percent were observed after only three hours.

There was the possibility that adrenal progesterone was somehow released by the LRF and that progesterone was responsible for the receptivity that was observed. But when the animals were adrenalectomized (Moss and McCann, 1973) they continued to show receptive behavior following the injection of LRF. Thus, LRF appears to be a releasing factor which, independent of its stimulatory action upon LH, is able to induce sexual receptivity in estrogen primed female rats. An earlier study had shown that hypophysectomized rats will continue to display receptive behavior ruling out an effect due to LH (Pfaff, 1973). LRF has also been shown to stimulate receptivity when implanted directly into the medial preoptic area of the hypothalamus (Verhulst and Herrenkohl, 1975).

It is of particular interest that LRF has been found primarily in those regions of the hypothalamus that have been implicated in the control of sexual receptivity. Regardless of whether investigators employed bioassay, radioimmunoassay or immunocytochemical techniques, the highest concentration of LRF has been consistently found in

axon terminals in the median eminence (Palkovits, Brownstein, Saavedra and Axelrod, 1974; Wheaton, Krulich and McCann, 1975; Setalo, Vigh, Schally, Arimura and Flerko, 1975; Barry, Dubois and Poulain, 1973). Although high concentrations of LRF have also been found in the arcuate nucleus (Palkovits et al., 1974), studies using immunocytochemical techniques have been unable to demonstrate the presence of LRF in arcuate cell bodies (Kordon, Kerdelhue, Pattou and Jutisz, 1974). These results have not been interpreted as evidence for the nonexistence of LRF neurons in this nuclei group since the levels of LRF in the perikarya could be below detectable levels. This possibility was suggested by Barry, Dubois and Carette (1974) in a study using an immunofluorescence technique to detect LRF in guinea pig brain. By using colchicine to block axoplasmic flow, they found LRF within cell bodies of arcuate neurons. This finding suggested that although LRF is synthesized in arcuate neurons, most of it is probably stored in axon terminals, thus accounting for undetectable amounts in the cell body. LRF has also been localized in the preoptic area, although recent studies have shown that most of the LRF is probably located in the organum vasculosum of the lamina terminalis (OVLT) (Kizer, Palkovits and Brownstein, 1976). This circumventricular organ is located just anterior to the optic chiasm and at the anterior extent of the third ventricle. The precise role that LRF in the OVLT plays in ovulation and LH release is not fully understood. Although early

lesion studies found a decline in LRF in the median eminence-arcuate region following lesions in the supra-chiasmatic region (Schneider, Crighton and McCann, 1969), more recent evidence would suggest that LRF in the OVLT is independent from LRF located in the medial basal hypothalamus (MBH). Deafferentation of the MBH using Halasz type knife cuts (Halasz, 1969) resulted in no change in LRF levels in the OVLT (Zimmerman, 1976). Kizer et al. (1976) reported evidence that such neuronal elements secreting LRF might be located in the medial preoptic area.

It is relevant that many of the areas involved in the secretion or control of LRF have been implicated directly in the control of sexual receptivity. Malsbury and Pfaff (1973) found that sexual behavior in ovariectomized rats could be stimulated by implants of estradiol in the medial preoptic area or the medial anterior hypothalamus. Similar findings had been reported earlier by Lisk (1962). Other studies using ablation procedures have shown that lesions in the medial portion of the anterior hypothalamus can reduce sexual receptivity (Clark, 1942) as do lesions in the ventromedial nucleus (Carrer, Asch and Aron, 1973/1974). However, lesions in the medial preoptic area of ovariectomized rats have been found to result in a reduction of the quantity of estrogen required to induce receptivity. The mean threshold of estradiol for lesioned animals was less than half the value required prior to lesioning (Powers and Valenstein, 1972). Conversely,

electrical stimulation of the medial preoptic area has been shown to result in a 47 percent reduction in the quality of lordosis (Napoli, Powers and Valenstein, 1972). Whether the implant, lesion and electrical stimulation effects are mediated by LRF cannot be determined based upon the existing literature.

If LRF is part of the mechanism by which the nervous system is able to modulate sexual receptivity, then compounds, such as prostaglandins which have been implicated in gonadotropin release, might also play a behavioral role. The results of several independent investigations have shown that certain prostaglandins are able to stimulate LH release in rats as well as in other species. Harms, Ojeda and McCann (1973; 1974) and Lau and Saksena (1974) found that PGE_2 , when injected either intravenously or subcutaneously, resulted in an increased release of LH in ovariectomized rats. PGE_1 , $PGF_{2\alpha}$ and $PGF_{1\alpha}$ were also found to have the same effect when ovariectomized rats were first primed with estrogen (Batta, Zanisi and Martini, 1974; Harms *et al.*, 1974). Confirmatory data is to be found in a number of additional reports (Spies, Norman and Campbell, 1973; Castracane and Saksena, 1974; Tsafriri, Koch and Lindner, 1973; Sato, Taya, Jyujyo, Hirono and Igarashi, 1974; Ratner, Wilson, Srivastava and Peake, 1974). An issue of dispute has been whether prostaglandins stimulate LH release by acting directly on the anterior pituitary gland or whether the stimulatory effect is exerted

at the level of the hypothalamus via the release of LRF.

Certain authors have reported that the stimulation of LH release following prostaglandin administration occurs as a result of prostaglandin stimulation of the pituitary gland. When delivered directly into the adenohypophysis, either in vitro or in vivo, certain prostaglandins were found to stimulate LH release (Sato et al., 1974). PGE₁ and PGE₂, at doses of 50 µg or 100 µg, resulted in a significant increase in plasma LH. PGF_{2α}, at the same concentrations, was ineffective in stimulating LH in this in vivo paradigm, however, it did stimulate LH release into the incubation medium in vitro. PGE₁ and PGE₂ were also effective in stimulating LH release from the incubated pituitaries. In contrast to the results reported by Sato et al. (1974), Sundberg, Fawcett, Illner and McCann (1975) reported no effect of either PGE₁, PGE₂, PGF_{1α} or PGF_{2α} on the release of LH from incubated pituitary glands. They did report an increase in intracellular cyclic-AMP, but this increase was associated with the release of growth hormone only.

A hypothalamic site of action had previously been suggested by Harms et al. (1974) when they found that PGE₂ stimulated LH release when given intravenously, but not when it was injected directly into the pituitary (A slight increase in plasma LH levels was found with pituitary injections of PGE₂ when the ovariectomized rats were first primed with estradiol benzoate, however.). On the basis of

these data, the authors concluded that the primary site of prostaglandin action is in the hypothalamus. Chobsieng, Naor, Koch, Zor and Lindner (1975) arrived at a similar conclusion after they found that intravenous PGE_2 resulted in a 120 percent increase in serum LH levels, but that PGE_2 failed to stimulate LH from incubated pituitaries. In addition, these same authors reported that anti-LRF serum prevented the LH release induced by PGE_2 . As assays for LRF were developed, it became possible to show unequivocally that PGE_2 can act directly at the level of the hypothalamus. When PGE_2 was injected into the third ventricle, there was a significant increase in the number of rats with detectable immunoassayable LRF (Ojeda, Wheaton and McCann, 1975a). The increase was noted at 1, 3 and 5 minutes after the injection. An increase in LRF concentration in hypophyseal portal plasma was also shown by Eskay, Warberg, Mical and Porter (1975) following the infusion of PGE_2 into the lateral ventricles of adult male rats. These authors also reported no effect of PGE_2 on LH release following infusion of the prostaglandin into the hypophyseal portal vessel. On the basis of these studies, it can be concluded that the primary site of action of prostaglandin stimulated LH release is in the hypothalamus, although under certain conditions, such as estrogen priming, direct stimulation at the level of the pituitary might also occur.

Additional evidence of prostaglandin involvement in LH release can be found in the results of studies in which

anti prostaglandin drugs were used (Orczyk and Behrman, 1972; Tsafriri, Koch and Lindner, 1973; Behrman, Orczyk and Greep, 1972; Armstrong and Grinwich, 1972; Ojeda, Harms and McCann, 1975b). In the Orczyk and Behrman (1972) study, aspirin and indomethacin, two drugs that can inhibit prostaglandin synthetase activity, were both found to decrease pituitary and hypothalamic levels of prostaglandin. These two drugs were also found to block ovulation, an effect that could be reversed by administration of LH or, in the case of aspirin, block by LRF (Orczyk and Behrman, 1972; Behrman et al., 1972). Since the ovulation block induced by indomethacin was not reversed with LRF or LH treatment, it suggested that most of the anti-ovulatory effects of indomethacin are mediated at the level of the ovary. In support of this conclusion, Tsafrini, Koch and Lindner (1973) demonstrated that although indomethacin was able to block ovulation in female rats, it had no effect on the proestrus surge of LH and continued to block ovulation when given after the proestrus LH surge. In vitro evidence that indomethacin might not act in the brain was suggested by the observation that 10 mg/kg of indomethacin does not influence the ability of brain homogenates to synthesize prostaglandins (Dembinska, Grodzinska and Piotrowicz, 1974). The same dose of indomethacin was found to depress prostaglandin synthesis in the lungs, kidneys and seminal vesicles. Not all of the evidence, however, supports a non nervous system site of action for indomethacin.

Evidence that indomethacin can act in the brain to block LH release has been found using both in vivo and in vitro paradigms. Ojeda et al. (1975b) either injected 10 μ g of indomethacin into the third ventricle of ovariectomized rats or implanted the drug into the medial basal hypothalamus using 30 gauge cannulas. In one to six hours, plasma LH levels were significantly depressed. Indomethacin was also found, in the same study, to block progesterone induced LH release in ovariectomized estrogen primed rats. However, in order for this effect to be observed, it was necessary to inject the drug subcutaneously 24 hours before the progesterone was injected. In vitro evidence in support of indomethacin exerting an effect on neural tissue was reported by Raffel, Clarenback, Peskar and Hertting (1976). In this study, it was found that indomethacin was able to inhibit prostaglandin release in particulate fractions from total rat brain homogenate.

The discrepancy as to where indomethacin exerts its inhibitory effect upon ovulation could be due in part to the fact that indomethacin has additional effects besides the inhibition of prostaglandin synthesis. For example, Sundberg et al. (1975) presented evidence that indomethacin was able to potentiate LRF stimulated gonadotropin release from the rat anterior pituitary. A similar potentiation of gonadotropin release with indomethacin was reported by Ojeda et al. (1975b). They found that indomethacin potentiated LRF stimulated FSH release in ovariectomized, estrogen

and progesterone treated rats. There is also evidence that indomethacin is able to block the uptake of dopamine and norepinephrine into synaptosomes (Clarenback et al. [unpublished] cited in Raffel et al., 1976).

The mechanisms by which prostaglandins stimulate the release of LH and LRF are only partially understood. Direct prostaglandin effects on the pituitary and possibly the brain are probably mediated by cyclic-AMP. A current theory of the mechanism of gonadotropin release is that releasing factors stimulate receptors on the surface of pituitary cells. Activation of adenylyl cyclase then occurs resulting in increased levels of cyclic-AMP. This initial step triggers a sequence of events leading to increased synthesis and release of pituitary hormones (McCann, 1971). Prostaglandins have been found to stimulate cyclic-AMP formation not only in peripheral tissues, but also in the brain and anterior pituitary (Wellman and Schwabe, 1973; Zor, Kaneko, Schneider, McCann, Lowe, Bloom, Borland and Field, 1969). Intravenous injections of PGE_2 resulted in a 72 percent increase in whole brain cyclic-AMP levels in mice. PGE_1 resulted in a 57 percent increase while $\text{PGF}_{2\alpha}$ was slightly less effective but still induced a significant increase in cyclic-AMP levels. When cyclic-AMP levels were measured in discrete regions of the rat brain following intravenous injections of PGE_2 , substantial increases were found in several brain regions and in the following order of magnitude: cerebral cortex, thalamus, hypothalamus,

hippocampus, striatum, brain stem and cerebellum (Wellman and Schwabe, 1973). Prostaglandin E_1 has also been found to stimulate adenylyl cyclase activity and increase cyclic-AMP levels in the anterior pituitary using in vitro techniques. Correlated with these increased levels of cyclic-AMP was an increased release of LH (Zor et al., 1969). Other investigators have reported similar correlations between PGE_1 and cyclic-AMP levels during the stimulation of LH release from the anterior pituitary (Makino, 1973).

The evidence suggesting a mechanism by which prostaglandins are able to stimulate LRF release is not as firm as it is for prostaglandin stimulated LH release. Although interactions between prostaglandins and neurotransmitters have been described (Weiner, Gorski and Sawyer, 1972; De Wied and de Jong, 1974; Brody and Kadowitz, 1974; Hinman, 1972), neurotransmitters do not appear to be involved in prostaglandin stimulation of LRF release. Harms, Ojeda and McCann (1976) used a variety of monoaminergic and cholinergic receptor blockers, but failed to block PGE_2 induced LH release. Since their pharmacologic manipulations appeared to preclude mediation by adrenergic, dopaminergic, serotonergic or cholinergic receptors, the authors suggested that PGE_2 acts directly at the level of the LRF neuron and not transsynaptically. Cells in the preoptic area and arcuate region of the hypothalamus have been shown to alter their

electrical discharge pattern following iontophoretic administration of prostaglandins (Poulain and Carette, 1974). There is additional evidence in sheep that the mechanism by which prostaglandins stimulate LRF neurons in the hypothalamus is part of the mechanism by which estrogen exerts its effects (Roberts and McCracken, 1975). They found that estradiol could modulate the amplitude of $\text{PGF}_{2\alpha}$ output from the brain and that $\text{PGF}_{2\alpha}$ levels increased at the time of the LH surge. If it is true that prostaglandins are part of the mechanism by which estrogen stimulates LRF, it is possible that prostaglandins are also part of the mechanism by which estrogen is able to stimulate sexual receptivity. The following experiments have been designed to test this possibility.

EXPERIMENT 1
EFFECTS OF INTRAVENTRICULAR
PROSTAGLANDIN E₂ ON SEXUAL RECEPTIVITY

Prostaglandins exert many effects upon reproductive physiology including mediation of luteinizing hormone releasing factor (LRF) action on LH (Collins, Elias, Henman, and Johnson, 1974; Harms, Ojeda, and McCann, 1974). But despite the voluminous literature documenting the effects of prostaglandins upon reproductive physiology, little is known of these compounds' effects upon reproductive behavior. One source of evidence suggesting that a behavioral role for these cyclic fatty acids does exist is the recent discovery that LRF can stimulate sexual receptivity in ovariectomized female rats primed with estrogen (Moss et al., 1975). Prostaglandin E₂ (PGE₂) injected directly into the third ventricle stimulates the release of LH, presumably via LRF (Chobsieng, Naor, Koch, Zor, and Lindner, 1975). Thus, since PGE₂ does act to stimulate LRF release, then it might also stimulate the display of sexual receptivity. The present study was designed to test this possible role of PGE₂ on sexual behavior as well as to assess its effects on body temperature and behavioral activity. The well established phenomena of prostaglandin induced fever was monitored to assess the release and biological activity of the implanted PGE₂. Behavioral activity levels

were measured as an indicant of the animals' overall activity levels since under certain conditions, PGE_2 has been shown to induce lethargy in some species (Gilmore and Shaikh, 1972).

Methods

Adult, female, CD albino rats were purchased from Charles Rivers Laboratories (Wilmington, MA). All animals were housed individually with food and water available at all times. The animal colony lights were left on from 2100 hr to 0900 hr. All behavioral testing and body temperature measurements were scheduled between 1100 and 1700 hr. The animals were ovariectomized under ether anesthesia and implanted with outer wall guide tubes under sodium pentobarbital anesthesia several weeks prior to the onset of the experiment. The 20 or 22 gauge outer wall tubes measured 5 mm in length and were placed stereotaxically into pre-drilled holes on midline overlying the anterior hypothalamus. Dental cement secured this guide tube to the skull. On the day of the tests, 27 gauge inner cannulas were tapped 20 times into either crystalline PGE_2 or cholesterol. These cannulas were constructed so that when placed into the outer guide tube the tip would be exposed to the anterior hypothalamus--third ventricle. At the end of each test day, the inner cannula was removed and inspected visually to verify that all of the PGE_2 had dissolved out. The procedure of implanting the PGE_2 or cholesterol was accomplished using an unanesthetized animal and required

approximately 1 minute of time. At the end of the experiment, the brains were fixed in 10 percent formal-saline, sectioned and stained for verification of the implant site.

Prior to the implant day, all animals received 1 μg of estradiol benzoate (E_2B) followed 48 hours later by 500 μg of progesterone. Only those animals showing receptivity scores of 60 or higher were used in the experiment. These animals were then once again primed with 1 μg of E_2B in preparation for the implants 2 days later. One hour before the cannulas were implanted, the animals were given a pre-test to insure that the estrogen priming by itself was not sufficient to induce receptivity. Animals showing receptivity scores of 50 or more were not used. Having met criteria on the E_2B plus progesterone and E_2B alone tests, the animals were then given dexamethasone (250 $\mu\text{g}/\text{kg}$) to reduce adrenal progesterone and hence the possibility that the subsequent repeated testing might enhance the animals' receptivity scores. Four sex tests were conducted at approximately hourly intervals following the implant. Activity was measured after the first and third sex tests. Body temperature was recorded following the second activity test. On the next day, all the animals were given 500 μg of progesterone and sex tests were conducted 3 hours later. This latter test was included in order to assess the effect of progesterone on receptivity following PGE_2 . Table 1 summarizes the treatment and test paradigm used in this experiment.

TABLE 1
Injection and Testing Paradigm
For Intraventricular PGE₂

Day 1:	1 µg E ₂ B
Day 2:	Nothing
Day 3:	500 µg Progesterone --(3 hr)-- Sex Test
Day 4:	1 µg E ₂ B
Day 5:	Nothing
Day 6:	Sex Pretest; Dexamethasone --(1 hr)-- Implant --(1 hr)-- S & A Tests* --(1 hr)-- Sex Test --(1 hr)-- S & A Tests: Body Temperature --(1 hr)-- Sex Test
Day 7:	500 µg Progesterone --(3 hr)-- Sex Test

*Sex and Activity Tests.

Behavioral tests were conducted in the animal colony under dim, red light illumination. All testing was done blind. For the sex tests, females were placed in 30 x 30 x 60-90 cm clear plexiglas testing arenas which contained previously habituated stud males. The floor of each arena was covered with a 1 to 2 cm layer of SAN-I-CEL bedding material. During the test, each female was mounted 10 times by the stud male. Only mounts with pelvic thrusts were counted. In response to each mount, the female was scored for the presence or absence of the receptive posture known as lordosis. The ratio of the number of lordotic responses to mounts times 100 (L/M or lordosis quotient) was used as the index of sexual receptivity. Activity tests were conducted in a 91.4 cm square clear plexiglas chamber with 16 equal sized squares, 4 per side. The animal to be tested was placed in the center of the chamber and the number of square crossings during the next 5 minutes was counted. A square crossing was recorded when three of the animal's feet had crossed a line into a given square. Rectal body temperature was recorded using a YSI Model 43 single channel telethermometer.

Because rectal stimulation has been shown to stimulate lordosis in ovariectomized female rats (Komisaruk, 1974), only one body temperature measurement was made on the day of sexual behavior testing. This was done following the third test so as to minimize any effects due to the thermal probe. Since other studies have reported a rise in body

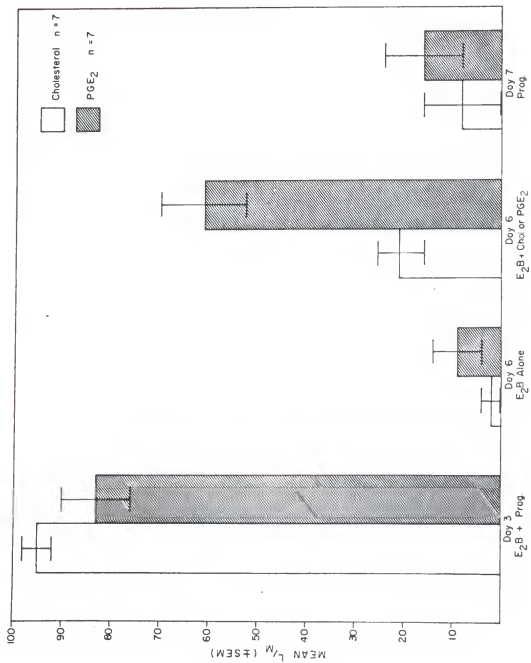
temperature within minutes after intracerebral PGE_2 administration (Potts and East, 1972), a second group of animals was tested only for body temperature and at 30 or 60 minute intervals beginning 30 minutes after the implantation. Some animals received PGE_2 while others received an implant of cholesterol or no implant at all. The body temperature data from the sex experiment animals and the temperature alone group were combined in Figure 2 and for statistical analysis.

Most of the animals served as their own controls. Females receiving PGE_2 on one week were retested the following week with cholesterol. Cholesterol animals were retested with PGE_2 . Animals failing to meet the various pretest criteria were either retested 3 weeks later or else used only on the week for which criteria was met.

Results

Intraventricular implants of PGE_2 were found to induce lordotic responses at levels significantly higher than under control conditions (Figure 1). Based upon the highest lordosis quotient exhibited on one of the four implant tests, animals receiving PGE_2 displayed a mean score of 63 while animals receiving cholesterol had a mean score of 33. These differences were significant at the $p < .01$ level ($t = 3.02$, $df = 13$). Because of the criteria used to select animals for implantation, all of the rats used exhibited high lordosis quotients on the E_2B and progesterone test and nonreceptive scores on the E_2B alone test.

FIGURE 1. Highest receptivity score exhibited on a given test day following intravenous implants of PGE₂ or cholesterol.



The L/M score following PGE₂ was not significantly different from that following E₂B and progesterone. There was no significant difference between the scores of the two groups when tested on day seven and three hours after progesterone priming. Neither the cholesterol nor PGE₂ animals had mean lordosis quotients of 50 or higher on this test.

There was no consistent trend regarding the time following the implants that the animals exhibited maximum receptivity. Each animal received four tests at approximately hourly intervals. However, variations in the amount of time that it took for the females to receive 10 mounts each and the blind testing procedure resulted in the animals actually getting tested at a variety of times. Table 2 lists the L/M scores for each animal and the time after the implant that the test was concluded. The highest score for each individual is underlined at the time point that it was first observed. At 165 minutes, all but one animal had responded with its highest score. Furthermore, the L/M score of the PGE₂ treated animals at this time point was significantly higher than the score displayed by the cholesterol animals at the 165 minute time point ($p < .05$, $t = 3.37$, $df = 6$). A single-factor analysis of variance revealed no significant differences across time and with the exception of the 165 minute tests, there were no other significant differences between the PGE₂ and control measures at specific testing intervals.

On the first activity test, the PGE₂ group had a mean

TABLE 2
Lordosis Quotient Over Time Following Intraventricular PGE₂ or Cholesterol

		Time After Implant (min.)									
Group	Animal	30	45	60	75	90	105	165	180	240	
PGE ₂	1		<u>70*</u>			70		60		40	
	3		<u>70</u>			30		40		20	
	5			10		30		<u>80</u>		60	
	6				10		<u>30</u>		30	0	
	7			20		10			<u>40</u>	10	
	9				<u>90</u>		80			0	
	13		10				<u>40</u>		40	40	
	15		30				70	<u>80</u>		70	
	$\bar{X} \pm \text{SEM}$	45 $\pm$ 15	15 $\pm$ 5	50 $\pm$ 40	35 $\pm$ 13	55 $\pm$ 12	65 $\pm$ 10	37 $\pm$ 3	30 $\pm$ 9		
Chol.	1	40		60				30		40	
	6		0			30			30	10	
	7		30				30	0		10	

9	30	10	40	20
12	10	0	30	0
13		0	10	20
15		20	20	0
$\bar{X} \pm \text{SEM}$	25 ± 15	20 ± 10	25 ± 5	23 ± 7
			30 ± 30	18 ± 10
				15 ± 15
				14 ± 5

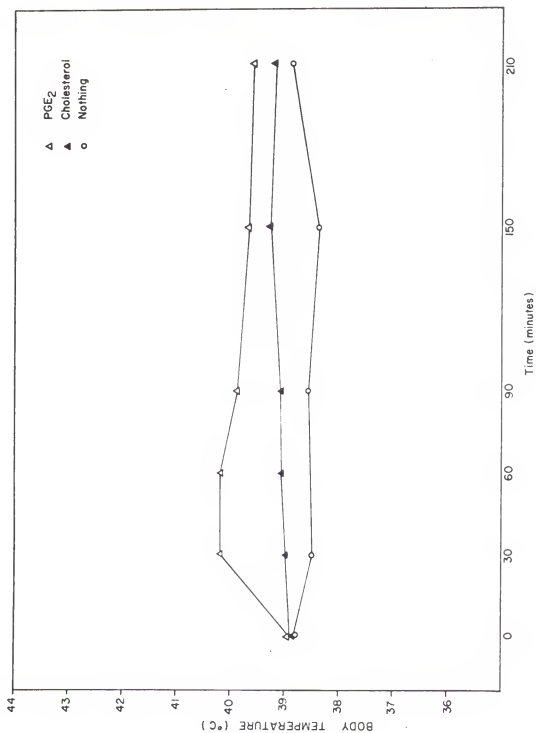
*Underlined score indicates the time point at which the animal's highest score was first exhibited on the PGE₂ test.

of 43 line crossings per five minutes, while the control animals were only slightly more active with an average of 58 line crossings. On the second activity test, the scores for the PGE₂ and control groups were 34 and 46 respectively. None of these differences were significant.

Body temperature was elevated in the PGE₂ group at the time the first measurement was made at 30 minutes (Figure 2). When the PGE₂ group was compared with the cholesterol and no-implant control groups, significant overall differences were found at 30, 60, 90 and 150 minutes. Newman-Keuls a posteriori comparisons using harmonic means revealed a significant difference between the PGE₂ and cholesterol groups at 30 minutes ($p < .01$) and at 90 minutes ($p < .05$). The PGE₂ group also had a greater hyperthermic response when compared to the nonimplanted group at 30 minutes ($p < .01$) and at 90 minutes ($p < .05$). There were no significant group differences at the time of the 150 minute measure. The only significant difference between the cholesterol and nonimplanted groups was at the 30 minute measure ($p < .05$).

Histological examination of the brains following the completion of the experiment revealed that the implants were either within the third ventricle or in the periventricular space. The implanted region extended from the septal area to the posterior hypothalamus.

FIGURE 2. Rectal body temperatures of animals receiving implants of either PGE₂, cholesterol or an empty cannula. All of the animals received 1 µg of E₂B two days prior to these measures. No measurement was made at 60 minutes of the control group receiving an empty cannula.



EXPERIMENT 2
INDUCTION OF SEXUAL RECEPTIVITY WITH PGE₂
IMPLANTS IN SPECIFIC SUB-CORTICAL REGIONS

Experiment 1 has suggested an excitatory role for PGE₂ upon sexual behavior in ovariectomized, estrogen primed female rats. The purpose of Experiment 2 was to determine whether the effect is more pronounced in specific sub-cortical regions. Three regions were selected based upon hypothesized mechanisms by which PGE₂ might be exerting its effect: the preoptic area (POA)-anterior hypothalamus, the medial basal hypothalamus (MBH) and the interpeduncular nucleus (IP). LRF, which also stimulates sexual behavior and is released by PGE₂, is under the influence of neurons in the POA as well as neurons in the MBH region of the hypothalamus (Zimmerman, 1976). These two regions also exhibit relatively high uptake of the behaviorally active hormone, estradiol (Luttge and Whalen, 1972). The IP was selected because it shows high uptake of tritiated progesterone, another hormone important in the mediation of sexual behavior (Whalen and Luttge, 1971a,b). Other studies have shown both facilitatory and inhibitory effects of progesterone implants in this region (Luttge, 1976; Hall and Luttge, 1974).

Methods

Twenty-four CD female rats weighing between 275 and

300 grams were bilaterally ovariectomized under ether anesthesia. They were allowed to have sexual experience with stud males after priming with 2 μ g sc E₂B followed 48 hours later by 500 μ g sc progesterone. After at least two such sequences of hormone priming and testing, all of the animals were anesthetized with sodium pentobarbital anesthesia (50 mg/kg) and implanted with 22 gauge outer wall guide tubes. These guide tubes measured approximately 5 mm in length and were attached by wires to skull screws before being encased in dental cement. Three such cannulas were stereotaxically placed in the calvarium overlying the POA, MBH and the IP. A recovery period of at least one week preceded the first behavioral test, at which time precisely measured inner wall cannulas of 28 gauge stock were lowered to the respective site. Cannulas were tapped 20 times into either crystalline PGE₂ or the control substance, cholesterol. Using this procedure, each cannula contained a mean $\pm$ SEM of 4 ± 1 μ g of PGE₂. At the end of the tests, the cannulas were removed and inspected visually to verify that the PGE₂ had dissolved out.

Each of the 24 animals was tested with both cholesterol and PGE₂ in each of the three sub-cortical regions over an 8 week period. Table 3 summarizes the treatment paradigm which called for dividing the animals randomly into 3 groups of $n = 8$. Each week, half of the animals in each group received cholesterol while the remaining half received PGE₂. The following week, the animals that had

TABLE 3
PGE₂ And Cholesterol Implantation Schedule

Week	Groups				
	A-1 Treatment	Site	A-2 Treatment	B-1 Treatment	Site
1	PGE ₂	POA	Cholesterol	PGE ₂	MBH
2	Cholesterol	POA	PGE ₂	Cholesterol	MBH
3	PGE ₂	IP	Cholesterol	PGE ₂	POA
4	Cholesterol	IP	PGE ₂	Cholesterol	POA
5	PGE ₂	MBH	Cholesterol	PGE ₂	IP
6	Cholesterol	MBH	PGE ₂	Cholesterol	IP

Table 3 - extended

B-2 Treatment	C-1 Treatment	Groups	
		Site	C-2 Treatment
Cholesterol	PGE ₂	IP	Cholesterol
PGE ₂	Cholesterol	IP	PGE ₂
Cholesterol	PGE ₂	MBH	Cholesterol
PGE ₂	Cholesterol	MBH	PGE ₂
Cholesterol	PGE ₂	POA	Cholesterol
PGE ₂	Cholesterol	POA	PGE ₂

previously received PGE_2 served as the controls and the initial control animals received PGE_2 . For the first two week test period, animals in Group A (see Table 3) received implants in the POA while Group B and C animals were tested with MBH and IP implants respectively. During weeks 3 and 4 and again during weeks 5 and 6, the implant site was changed for each group so that after 6 weeks, each of the animals had been tested with the implant in all 3 sites.

The daily injection and testing sequence is summarized in Table 4. On day 1, all animals were tested following treatment with 2 μg sc E_2B (48 hr prior to this test) and 500 μg progesterone (3 hr prior to this test). On day 2, all animals were primed with the same dose of E_2B . Forty-eight hours later, on day 4, animals were tested with stud males 30 minutes after the offset of the light cycle (0930 hr). Upon completion of this behavioral test, which was terminated after each animal had been mounted 10 times by a stud male, each animal was implanted. Rectal body temperature was recorded just prior to and again 15 minutes after the PGE_2 or cholesterol had been implanted. A second sex test was conducted 3 hours after implantation. Immediately after this second sex test, activity levels were measured using an open field activity chamber. The total number of line crossings occurring during the 5 minute test was used as the activity measure. On day 6, all animals were again primed with E_2B followed 48 hours later (day 1 of the subsequent week) by progesterone. Further details

TABLE 4
Injection and Testing Paradigm
For Intracerebral PGE₂

Day 1:	500 µg Progesterone --(3 hr)-- Sex Test
Day 2:	2 µg E ₂ B
Day 3:	Nothing
Day 4:	Sex Test Body Temperature Implant Body Temperature --(3 hr)-- S & A Tests*
Day 5:	Nothing
Day 6:	2 µg E ₂ B
Day 7:	Nothing

*Sex and Activity Tests.

of the testing paradigm are described in the methods section of Experiment 1.

Results

Gross and microscopic examination of serial sections through the brains of all of the animals necessitated dividing the rats into two groups based upon histology. For each of the three regions, it was determined whether the cannula tip was located within the neuropil or whether it had extended out of the brain and into the sub-arachnoid space. Data from each of these two subgroups were analyzed separately since diffusion through the CSF in the sub-arachnoid space could have resulted in the PGE₂ having exerted its effects in brain areas distal to the immediate vicinity of the implant site. In some of the animals, the cannula tract in a given brain region could not be found. Data from these animals were not included in the statistical analysis. The decision was also made to exclude those animals that displayed lordosis quotients of 50 or higher following the cholesterol implants. However, since this criteria was established a posteriori, Table 5 includes a separate analysis in which data from animals failing to meet this criteria were analyzed. Appendix Figure A-1 summarizes the sites of the cannula tips for the animals used in Experiment 2.

In those animals with cannula tips located within the neuropil, PGE₂ resulted in L/M scores that were significantly higher than cholesterol scores in the POA ($p < .05$,

TABLE 5
Individual Lordosis Quotient Scores Following Intracerebral PGE₂ And Cholesterol (Chol)

	Animal	POA PGE ₂	Implant Region				IP PG	Chol
			Animal	MBH PG	Animal	Chol		
Neuropil	*1	100	2	80	6	0	50	0
	2	80	3	80	8	40	20	0
	3	90	6	0	*19	0	0	nt†
	4	40	7	0	20	40	10	0
	8	100	9	0	23	30	0	0
	*9	60	16	100	25	20	10	0
	*13	100	17	20	28	0	80	0
	18	90	19	20	*30	40	80	70
	23	90	*23	0		nt†		
	25	90	25	90		40		
	30	90	*26	100		70		
$\bar{X} \pm \text{SEM}$ with *		85 ± 6		45 ± 13		28 ± 7	31 ± 12	10 ± 10
$\bar{X} \pm \text{SEM}$ without *		84 ± 7		43 ± 14		23 ± 6	28 ± 12	0 ± 0

Sub- Arachnoid Space	*7	20	100	8	80	0	3	30	20
	*10	30	nt†	13	80	0	4	0	0
	11	0	0	*14	60	90	14	70	30
	*14	100	70	20	90	40	16	0	0
	17	0	0	29	20	0	17	30	0
	*20	90	nt†				21	0	0
	26	0	30						
	28	70	40						
$\bar{X} \pm \text{SEM}$ with *	39 $\pm$ 15	40 $\pm$ 16			66 $\pm$ 12	26 $\pm$ 18		22 $\pm$ 11	8 $\pm$ 5
$\bar{X} \pm \text{SEM}$ without *	18 $\pm$ 18	18 $\pm$ 10			68 $\pm$ 16	10 $\pm$ 10		22 $\pm$ 11	8 $\pm$ 5

*Animals that had L/M scores of 50 or higher on cholesterol test or that were not tested with a cholesterol implant.

†nt Animal not tested or score not included because of failure to meet criteria on E₂B alone or E₂B plus progesterone tests.

$t = 2.42$, $df = 7$), MBH ($p < .05$, $t = 2.85$, $df = 8$) and IP ($p < .01$, $t = 4.58$, $df = 5$). A single-factor analysis of variance followed by a Newman-Keuls a posteriori test using harmonic means also revealed that PGE₂ implants in the POA elicited L/M scores that were significantly higher than L/M scores elicited by PGE₂ in the MBH ($p < .05$) and IP ($p < .01$). However, there was no significant difference between the MBH and IP scores. The same differences and levels of significance were found when the analysis included the animals that showed lordosis quotients of 50 or higher on the cholesterol test (Table 5).

An analysis of variance revealed no significant differences between the cholesterol scores for the three brain areas when the animals with high scores were excluded from the analysis. There was, however, an overall effect ($p < .05$) when these animals were included. Cholesterol scores when the cannula tip was within the neuropil in the MBH were significantly higher than the cholesterol scores following the IP implant ($p < .05$, Newman-Keuls test).

No significant differences were found following analysis of data from animals with implants in the sub-arachnoid space. There was no overall effect across brain regions and paired-t tests revealed no differences between the treatments (Table 5). However, the mean lordosis quotient following PGE₂ implants into the sub-arachnoid space below the MBH was nonsignificantly higher than either the POA or IP sub-arachnoid implants. It was also higher than the

scores following implants of PGE_2 within the neuropil of the MBH and IP.

The low L/M scores found with implants within the MBH and IP could not be correlated with low activity scores. No significant differences in activity scores were found across brain regions or between treatments regardless of whether the implants were within the neuropil or in the sub-arachnoid space (Table 6). If any trend was apparent, it was in the opposite direction since POA implants of PGE_2 resulted in fewer line crossings compared to the MBH and IP implants.

Compared with the preimplant values, PGE_2 implants resulted in a significant hyperthermic response in both the POA ($p < .001$, $t = 8.73$, $df = 7$) and the MBH ($p < .001$, $t = 7.23$, $df = 8$) (Table 7). No significant elevation in body temperature was observed following implants in the IP when the cannula tip was within the neuropil. But when the cannula extended into the sub-arachnoid space below the IP, a significant rise in body temperature was observed ($p < .05$, $t = 2.58$, $df = 5$). No elevation in body temperature was observed when cholesterol was implanted into the MBH or IP (Table 8). However, the control implants did result in a significant hyperthermic response in the POA ($p < .01$, $t = 4.64$, $df = 7$).

TABLE 6
Individual Activity Scores Following Intracerebral PGE₂ or Cholesterol (Chol)

Implant Region									
POA		Chol		Animal		MBH			
Animal	PGE ₂						PGE ₂	Chol	
Neuropil									
*1	1	7	2	92	95	6	70	63	Chol
2	118	94	3	63	34	8	49	66	
3	39	82	6	112	142	*19	99	nt†	
4	3	45	7	114	83	20	83	57	
8	46	15	9	59	5	23	0	65	
*9	113	26	16	106	66	25	286	60	
*13	13	19	17	34	60	28	39	1	
18	56	88	19	52	31	*30	42	24	
23	56	49	*23	105	41				
25	47	145	25	65	92				
30	55	33	*26	94	141				
X±SEM with *									
	50±12	55±13		81± 8	72±13		84±31	48±10	
X±SEM without *									
	53±11	69±15		77±10	68±14		88±41	52±10	

Sub- Arachnoid Space	*7	12	9	8	15	2	3	53	34
	*10	25	76	13	11	68	4	131	44
	11	57	26	*14	99	25	14	78	101
	*14	80	51	20	50	80	16	7	87
	17	52	9	29	63	72	17	50	86
	*20	27	120				21	42	23
	26	22	98						
	28	46	36						
$\bar{X} \pm \text{SEM}$ with *		40 $\pm$ 8	53 $\pm$ 15		48 $\pm$ 16	49 $\pm$ 15		60 $\pm$ 17	63 $\pm$ 13
$\bar{X} \pm \text{SEM}$ without *		44 $\pm$ 8	42 $\pm$ 19		35 $\pm$ 13	56 $\pm$ 18		60 $\pm$ 17	63 $\pm$ 13

*Animals that had L/M scores of 50 or higher on cholesterol test or that were not tested with a cholesterol implant.

†nt Animal not tested or score not included because of failure to meet criteria on E₂B alone or E₂B plus progesterone tests.

TABLE 7
Individual Rectal Body Temperature Measures Before And After Intracerebral PGE₂

		Implant Region							
		POA		MBH		IP			
Animal		Pre	Post	Animal	Pre	Post	Animal	Pre	Post
Neuropil	*1	38.5	40.3	2	39.0	39.6	6	39.4	39.2
	2	38.9	40.4	3	38.4	39.8	8	38.9	40.7
	3	38.5	40.5	6	39.0	40.4	*19	39.7	39.3
	4	39.3	40.2	7	39.1	40.5	20	39.3	40.6
	8	39.1	41.0	9	38.7	39.4	23	39.3	38.8
	*9	38.4	40.4	16	39.0	41.1	25	39.3	40.9
	*13	38.8	40.6	17	39.1	41.3	28	39.3	39.8
	18	39.6	40.8	19	39.2	41.7	*30	39.5	40.6
	23	39.3	40.0	*23	39.3	41.2			
	25	39.6	41.0	25	39.6	41.4			
	30	39.4	41.0	*26	39.5	41.0			
$\bar{X} \pm \text{SEM}$ with *		39.0 $\pm$ 0.1	40.6 $\pm$ 0.1		39.1 $\pm$ 0.1	40.7 $\pm$ 0.2		39.3 $\pm$ 0.1	40.0 $\pm$ 0.3
$\bar{X} \pm \text{SEM}$ without *		39.2 $\pm$ 0.1	40.6 $\pm$ 0.1		39.0 $\pm$ 0.1	40.6 $\pm$ 0.3		39.3 $\pm$ 0.1	40.0 $\pm$ 0.4

Sub-Arachnoid Space	*7	39.4	41.0	8	38.8	41.1	3	38.8	40.9
	*10	39.2	41.4	13	39.1	40.6	4	39.0	40.5
	11	39.5	41.3	*14	38.9	40.2	14	38.8	41.2
	*14	38.8	39.6	20	39.4	41.1	16	39.9	39.0
	17	39.0	40.8	29	39.2	40.7	17	38.8	39.7
	*20	39.4	41.5				21	39.0	40.4
	26	39.2	41.4						
	28	40.2	41.5						
$\bar{X} \pm \text{SEM}$ with *		39.3 $\pm$ 0.1	41.1 $\pm$ 0.2		39.1 $\pm$ 0.1	40.7 $\pm$ 0.2		39.1 $\pm$ 0.2	40.3 $\pm$ 0.3
$\bar{X} \pm \text{SEM}$ without *		39.5 $\pm$ 0.3	41.3 $\pm$ 0.2		39.1 $\pm$ 0.1	40.9 $\pm$ 0.1		39.1 $\pm$ 0.2	40.3 $\pm$ 0.3

*Animals that had I/M scores of 50 or higher on cholesterol test or that were not tested with a cholesterol implant.

TABLE 8
Individual Rectal Body Temperature Measures Before And After Intracerebral Cholesterol

	Animal	POA		Implant Region				IP
		Pre	Post	Animal	MBH	Post	Animal	Pre
Neuropil	*1	38.0	38.9	2	39.0	39.3	6	38.8
	2	38.1	39.3	3	38.7	39.0	8	38.9
	3	38.5	39.7	6	38.8	39.2	20	39.3
	4	39.1	39.7	7	39.2	39.3	23	39.2
	8	38.9	39.4	9	38.9	39.1	25	39.7
	*9	38.7	39.2	16	39.0	39.1	28	39.5
	*13	37.9	40.6	17	38.7	39.5	*30	39.3
	18	39.7	39.9	19	38.8	38.6		37.9
	23	39.4	40.0	*23	39.8	39.3		
	25	39.7	40.0	25	39.6	39.1		
$\bar{X} \pm \text{SEM}$ with *	30	39.5	39.9	*26	39.0	39.3		
		38.9 $\pm$ 0.2	39.7 $\pm$ 0.1		39.0 $\pm$ 0.1	39.2 $\pm$ 0.1		38.9 $\pm$ 0.2
		39.1 $\pm$ 0.2	39.7 $\pm$ 0.1		39.0 $\pm$ 0.1	39.1 $\pm$ 0.1		39.0 $\pm$ 0.2
$\bar{X} \pm \text{SEM}$ without *								

Sub-Arachnoid Space	*7	39.4	39.7	8	38.7	39.1	3	38.8	38.4
	*10	39.3	40.2	13	39.1	39.5	4	39.6	39.4
	11	39.2	40.3	*14	39.0	39.3	14	38.8	38.7
	*14	39.4	39.2	20	39.8	39.5	16	39.1	39.8
	17	38.8	39.2	29	39.2	39.3	17	39.1	38.9
	*20	39.4	39.1				21	38.8	38.5
	26	39.2	40.4						
	28	39.0	39.8						
$\bar{X} \pm \text{SEM}$ with *		39.2 $\pm$ 0.1	39.7 $\pm$ 0.2		39.2 $\pm$ 0.2	39.3 $\pm$ 0.1		39.0 $\pm$ 0.1	39.0 $\pm$ 0.2
$\bar{X} \pm \text{SEM}$ without *		39.1 $\pm$ 0.1	39.9 $\pm$ 0.3		39.2 $\pm$ 0.2	39.4 $\pm$ 0.1		39.0 $\pm$ 0.1	39.0 $\pm$ 0.2

*Animals that had L/M scores of 50 or higher on cholesterol test or that were not tested with a cholesterol implant.

EXPERIMENT 3
EFFECTS OF SYSTEMIC AND INTRACEREBRAL INDOMETHACIN ON
ESTRADIOL AND PROGESTERONE INDUCED SEXUAL RECEPTIVITY

The results of Experiments 1 and 2 have provided firm evidence that PGE_2 can induce sexual receptivity in estradiol primed ovariectomized rats. This cyclic fatty-acid was subsequently found to stimulate highest levels of receptivity when implanted in the POA and anterior hypothalamic region. Although this same general area has been implicated by a variety of techniques in the mediation of estrogen and progesterone induced sexual receptivity, the results of Experiments 1 and 2 do not provide any evidence that estrogen and progesterone might exert their behavioral effects via PGE_2 .

In order to determine whether or not prostaglandin synthesis is necessary for the display of estrogen and progesterone induced receptivity, a third group of animals was treated with either sc or intracerebral indomethacin. Indomethacin has been shown to block the action of prostaglandin synthetase which converts arachidonic acid to both PGE_2 and $\text{PGF}_{2\alpha}$ (Ferreira and Vane, 1974). It was hypothesized that if prostaglandin synthesis is necessary for the display of sexual receptivity, then the administration of this drug during estrogen and progesterone priming should block the display of sexual behavior.

Part A Methods

Twenty female CD rats were purchased from Charles Rivers Laboratories for this study. The animals weighed between 275 and 325 grams and were housed one per cage on the same 12:12 hour reversed light cycle described in Experiment 1. All of the animals were bilaterally ovariectomized prior to receiving their first hormone injections. Baseline levels of receptivity were established by testing the animals after 1 to 4 μg sc E_2B followed 48 hours by 500 μg progesterone.

After at least three such tests, 16 animals having displayed lordosis quotients of 50 or higher were matched and assigned to either the indomethacin ($n = 8$) or control group ($n = 8$). Table 9 summarizes the treatment and test paradigms for all of the indomethacin studies. In this first indomethacin experiment, the animals were treated with 1 μg of E_2B (day 1) followed 48 hours later on day 3 with 500 μg of progesterone. In addition to these steroids, animals in the experimental group received 3 daily sc injections of 5 mg indomethacin in 0.1 cc of 0.1 M sodium phosphate buffered saline starting on day 1. Control group animals received the same volume of vehicle. A total of 15 mg of indomethacin was injected by the time that the behavioral tests were conducted. Three hours after the progesterone injections on day 3, all of the animals were tested for sexual receptivity and open field activity levels. Both of these tests were conducted in the same

TABLE 9
Injection and Testing Paradigm For Indomethacin Experiments

Day	Experiment 3A	Experiment 3B
1	1 μ g E ₂ B: 5 mg Indomethacin	3 μ g E ₂ B
2	5 mg Indomethacin	2.5 mg Indomethacin
3	500 μ g Progesterone: 5 mg Indomethacin: Body Temperature --(3 hr)-- S & A Tests*: Body Temperature	Sex Pre-test: 500 μ g Progesterone: 2.5 mg Indomethacin: Body Temperature --(3 hr)-- S & A Tests: Body Temperature

*Sex and Activity Tests.

Table 9 - extended

Experiment 3C

2 μ g E₂B: 2.5 mg Indomethacin

2.5 mg Indomethacin

Sex Pre-test:

500 μ g Progesterone:

2.5 mg Indomethacin:

Body Temperature

-- (3 hr) --

S & A Tests:

Body Temperature.

manner as was described in Experiment 1. Rectal body temperature was also measured. The first measure was made immediately prior to the behavioral tests, while the second measure was made immediately after the tests.

Part A Results

The mean lordosis quotient of animals receiving 15 mg of systemic indomethacin was 16 compared to 65 for the control animals (Figure 3). This difference was significant ($p < .05$, $t = 2.83$, $df = 14$). Although the animals were nonreceptive following indomethacin, they were also extremely lethargic (Figure 4). Animals treated with the drug had a mean of 15 line crossings during the open field activity tests compared to 51 line crossings for the control animals. This difference was significant ($p < .05$, $t = 2.76$, $df = 18$) (Figure 4).

Indomethacin treated animals also had a pretest mean body temperature that was significantly lower than the pretest values for both the vehicle control group and a non-treated E₂B plus progesterone treated group ($p < .01$, Newman-Keuls test).

The reduced L/M and activity scores were most likely the result of the extremely poor health of the indomethacin treated animals. After 3 days of treatment, all of the animals had severe diarrhea and within 4 days after the final injection of the drug, 7 of the 8 treated animals were dead. Although body weights had not been recorded prior to the administration of the indomethacin, on the day

FIGURE 3. Receptivity scores of rats receiving 5, 7.5 or 15 mg of indomethacin injected sc or 30 μ g of crystalline indomethacin implanted into the anterior hypothalamus. Open bars represent scores following control injections of vehicle or implantation of empty cannulas.

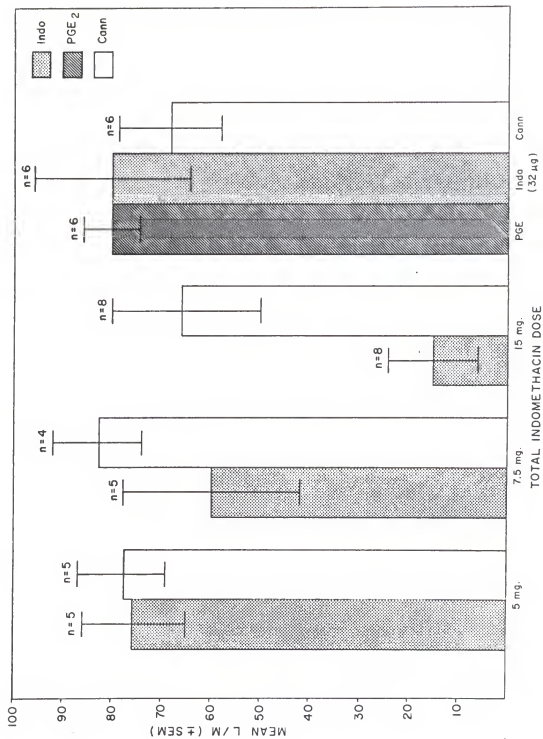
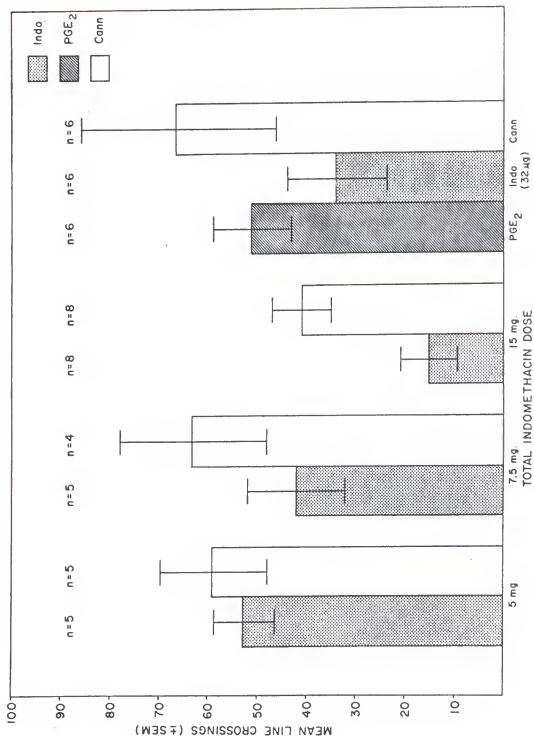


FIGURE 4. Open field activity scores of rats receiving 5, 7.5 or 15 mg of indomethacin injected sc or 30 μ g of crystalline indomethacin implanted into the anterior hypothalamus. Open bars represent control scores following either the injection of the sc vehicle of the implantation of an empty cannula.



of the tests the mean weight of the treated animals (296 g) was significantly lower than the mean weight of the control group (331 g) ($p < .01$, $t = 3.72$, $df = 17$).

Part B Methods

Ten CD ovariectomized rats were used in this experiment. Eight of the animals were from the control group of Experiment 3A while two additional animals were from a separate population of ovariectomized female rats that had a similar history of E_2B and progesterone priming. All of the animals received additional baseline tests to establish the minimum dose of E_2B capable of inducing receptivity with progesterone, but not with E_2B alone.

The two doses of indomethacin used in this experiment were 5 mg total and 7.5 mg total (Table 9). For the test using the lower dose, 2.5 mg was administered in .05 cc of the phosphate buffered vehicle. The drug or vehicle injections were given only on days 2 and 3 for a total of 5 mg. A week later, the drug was injected at the same concentration, however, it was also given on the day of E_2B priming (day 1) for a total dose of 7.5 mg.

The testing schedule was the same as described for Experiment 3A with the addition of a sex pretest conducted on day 3 just prior to injecting the progesterone (Table 9). This test was carried out to ascertain that the E_2B dose by itself was insufficient to induce receptivity.

Part B Results

Neither the 5 mg nor the 7.5 mg total doses of

indomethacin was able to significantly inhibit lordotic behavior in estrogen and progesterone primed rats (Figure 3). The mean L/M for the 5 mg indomethacin group was 76 compared to 78 for the control animals. A slight reduction in the level of lordosis was observed when the indomethacin dose was increased to 7.5 mg. Treated animals displayed a mean lordosis quotient of 60, compared to 83 for the control animals. None of these differences for the 5 or 7.5 mg doses were significant, however.

Unlike the results following 15 mg of indomethacin, no significant reduction in activity levels was observed in either the 5 or 7.5 mg groups (Figure 4). However, the actual scores suggested that the nonsignificant decrease in the lordosis quotient might have been correlated with a nonsignificant decrease in activity levels. Animals receiving 5 mg of indomethacin displayed an average of 53 line crossings compared to 59 for the 5 mg dose control animals. When the dose was increased to 7.5 mg, the treated animals had a mean of 42 line crossings compared to 63 for their controls. Although the health of the animals appeared normal following the 5 mg dose of indomethacin, 2 of the 5 animals that received the 7.5 mg dose died one week after the final injection. At the time of both tests, all of the animals appeared to be in good health. However, it is possible that the slightly reduced L/M and activity scores during the 7.5 mg test were due to a health problem that had not yet manifested itself through the animals appearance and behavior.

Neither dose of indomethacin had any effect upon rectal body temperature (Table 10). The mean values for the 5 mg indomethacin and control animals was 38.7°C and 38.5°C respectively. For the 7.5 mg treated animals, the indomethacin resulted in a mean temperature of 38.7°C while the control animals were measured at 39.1°C.

Part C Methods

Animals used in this experiment were selected from a separate population of 20 CD female rats purchased from Charles Rivers Laboratories. Upon their arrival, the animals were bilaterally ovariectomized under ether anesthesia. One week later, the animals were implanted bilaterally with 22 gauge outer walled guide tubes over the POA and anterior hypothalamus. The procedure used to implant these outer walled guide tubes was the same as described in Experiments 1 and 2. On the day of the tests, 28 gauge stainless steel cannulas were tapped 20 times into crystal-line indomethacin and then implanted bilaterally into the 22 gauge guide tubes. Empty cannulas were implanted into the rats during the control test. Using this implant procedure, a total of $32 \pm 2 \mu\text{g}$ of indomethacin was administered to each animal. Upon their removal at the end of the behavioral tests, the cannulas were inspected visually to verify that all of the drug had dissolved out.

All of the animals were tested at least 4 times with systemic E_2B and progesterone to determine individual threshold doses of E_2B . Some animals with higher thresholds

TABLE 10
 Mean $\pm$ SEM Rectal Body Temperature ($^{\circ}$ C)
 Following Systemic And Intracerebral Indomethacin

Indomethacin Dose	Pre-test	Post-test
5 mg	--	38.7 $\pm$ 0.1
Control	--	38.5 $\pm$ 0.2
7.5 mg	--	38.7 $\pm$ 0.2
Control	--	39.1 $\pm$ 0.1
15 mg	36.6 $\pm$ 0.2	37.3 $\pm$ 0.2
Control	38.0 $\pm$ 0.2	39.3 $\pm$ 0.2
Treatment	Pre-implant	Post-implant
PGE ₂ Implant	39.1 $\pm$ 0.2	40.1 $\pm$ 0.3
Indomethacin Implant	37.9 $\pm$ 0.2	39.5 $\pm$ 0.3
Empty Cannula	37.6 $\pm$ 0.2	39.3 $\pm$ 0.4

received additional tests. When the animals were displaying L/M scores of 50 or higher during these baseline tests, they were used in the experiment. Doses of E_2B used for this study ranged from 2 to 8 μg . Prior to receiving indomethacin, all of the animals were implanted either bilaterally or unilaterally with PGE_2 . This test was conducted in order to insure that the outer guide tubes were positioned over an area of the brain where implants of PGE_2 would induce receptivity.

On the day of the tests, the rats were implanted bilaterally with indomethacin or cholesterol 2 hours prior to the injection of progesterone and 5 hours prior to the sex test. Rectal body temperature was measured just prior to the implant procedure and again at 15 and 120 minutes afterwards. Sexual receptivity and open-field activity tests were conducted 3 hours after progesterone injection. At the conclusion of the behavioral tests, a final body temperature reading was made. One week later, the animals were retested using the same procedure except that control animals were tested with indomethacin and indomethacin animals received an empty cannula on the second test.

Part C Results

The original design of this study called for implanting PGE_2 bilaterally into the POA. This test was to establish whether or not the indomethacin implants were in a region of the brain where PGE_2 implants could induce receptivity. But in marked contrast to the previous experiments using unilateral implants of PGE_2 , the bilateral

paradigm used in a total of 7 animals proved lethal. Within 3 hours after implantation, activity scores were drastically reduced to 23 line crossings compared to 63 for a group of unilaterally implanted animals ($p < .01$, $t = 3.45$, $df = 14$). The mean lordosis quotient for the bilaterally implanted animals was 14. This value can be attributed to a single animal that had a lordosis quotient of 100. The same animal had a relatively high activity score with 75 line crossings, more than double the score of any other individual. Five of the animals with reduced receptivity and activity scores were observed to be suffering from severe neurologic symptoms as manifested by ataxia and loss of the righting reflex. Blood was observed in the region around the eyes. Within approximately 6 hours, 4 of the 7 implanted animals were dead. Gross examination of their brains revealed massive intracerebral hemorrhage of greatest extent in the immediate vicinity of the implant site.

A subsequent group of animals ($n = 14$) was tested with a unilateral implant of PGE_2 in the POA. The mean L/M for this test was 59. Of the 10 animals that had L/M scores of 50 or higher on the PGE_2 test, 6 were eventually used for the indomethacin implant test. The remaining 4 were not used due to clogged guide tubes at the time of the drug tests. The mean L/M score for this group of 6 animals was 80 following unilateral implants of PGE_2 (Figure 3). When indomethacin was implanted bilaterally into this same area, the lordosis quotients for these same animals remained

elevated at 80. Although the mean L/M score was slightly lower at 68 following the empty cannulas, this value was not significantly lower than the lordosis quotients observed after PGE₂ or indomethacin.

When the data analysis included animals that had not been receptive following PGE₂ implants, there was still no significant effect due to indomethacin. Indomethacin treated animals had a mean L/M score of 68 compared to 55 for the control condition.

There was no significant difference between the activity scores resulting from the indomethacin implants and the control condition when data from all of the animals were analyzed. But when the analysis was based only upon those animals that had shown lordotic responding of 50 or higher following PGE₂ implants, a significant difference was observed (Figure 4). Indomethacin resulted in a significant decrease in the mean number of line crossings compared with the control condition ($p < .05$, $t = 3.00$, $df = 5$). However, this decrease in activity was not correlated with a decrease in sexual responsiveness as was the case in the experiments using systemic injections of indomethacin.

A significant hyperthermic response was observed following both the implants of indomethacin as well as empty cannulas when the pre- and postimplant temperatures were compared ($p < .001$) (Table 10). However, there was no difference between the postimplant temperature following indomethacin and the postimplant temperature following the

empty cannula. Both of these values were significantly lower than the temperature measured following the implants of PGE_2 into all of the animals. No significant differences were observed, however, when the data analysis was limited to those animals that were receptive on the PGE_2 test.

EXPERIMENT 4
EFFECTS OF ADRENALECTOMY ON
PGE₂ INDUCED SEXUAL RECEPTIVITY

A mechanism by which PGE₂ could exert its facilitatory effects upon sexual behavior is via stimulation of adrenal progesterone. This possibility is quite viable, especially since the prostaglandin synthetase inhibitor indomethacin failed to inhibit estradiol and progesterone induced receptivity (Experiment 3). This suggests that PGE₂, though able to stimulate receptivity, is not part of the mechanism by which estradiol and progesterone exert their effects. Experiment 4 was conducted in order to assess the role played by the adrenal glands in PGE₂ stimulated sexual receptivity.

Methods

Six rats, having shown L/M scores of 50 or higher following intracerebral PGE₂ during Experiment 3C underwent bilateral adrenalectomy. Over several weeks, the animals received repeated sex and activity tests following a variety of treatment paradigms summarized in Table 11. The first test was conducted after E₂B followed 48 hours by implanted PGE₂ (E+PG-1). All of the animals were then tested with E₂B followed 48 hours by progesterone (E+PR-1) and then one more time with implants of PGE₂ (E+PG-2). Test 4 following adrenalectomy was conducted after the

TABLE 11
Injection and Testing Paradigm Following Adrenalectomy

	Group	
Test 1	E+PG-1	E ₂ B†--(48 hr)-- PGE ₂ Implant --(3 hr)-- S & A Tests*
Test 2	E+PR-1	E ₂ B --(48 hr)-- 500 µg Progesterone --(3 hr)-- S & A Tests*
Test 3	E+PG-2	E ₂ B --(48 hr)-- PGE ₂ Implant --(3 hr)-- S & A Tests
Test 4	E+PR+PG	E ₂ B --(48 hr)-- 500 µg Progesterone: PGE ₂ Implant --(3 hr)-- S & A Tests
Test 5	E+PR-2	E ₂ B --(48 hr)-- 500 µg Progesterone --(3 hr)-- S & A Tests
Test 6	E+CS+PG	E ₂ B --(48 hr)-- 250 µg Corticosterone --(30 min)-- PGE ₂ Implant --(2 hr)-- 250 µg Corticosterone --(1 hr)-- S & A Tests
Test 7	E+PR-3	E ₂ B --(48 hr)-- 500 µg Progesterone --(3 hr)-- S & A Tests
(Pre-adrenalectomy)	E	E ₂ B --(48 hr)-- Sex Test
(Pre-adrenalectomy)	E+PG-ad	E ₂ B --(48 hr)-- PGE ₂ Implant --(3 hr)-- S & A Tests

†Doses varied between 4 µg and 8 µg depending upon individual thresholds.

*Sex and Activity Tests.

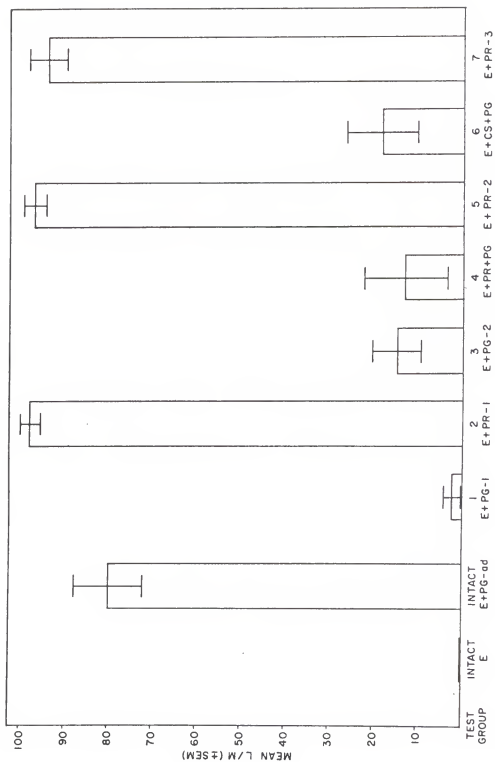
standard E₂B and progesterone paradigm, but in addition, the animals received implants of PGE₂ 3 hours prior to the tests and concurrently with the progesterone injections (E+PR+PG). On test 5 the animals were tested following E₂B and progesterone (E+PR-2). On test 6, the animals were primed with E₂B and 48 hours later, implanted with PGE₂. Thirty minutes prior to the implants and again one hour before the tests, the animals received sc injections of 250 µg/animal of corticosterone suspended in saline (E+CS+PG). A final test was carried out following E₂B and progesterone (E+PR-3). These postadrenalectomy scores were compared to preadrenalectomy scores for the same animals following E₂B alone (E) and E₂B priming followed by implantation of PGE₂ (E+PG-ad).

Results

Adrenalectomy was found to block PGE₂ induced receptivity (Figure 5). A single-factor analysis of variance for repeated measures followed by a Newman-Keuls a posteriori test revealed the following group differences: (E+PR-1), (E+PR-2), (E+PR-3) > (E+CS+PG), (E+PG-1), (E+PG-2), (E+PR+PG), (E) ($p < .01$). Although PGE₂ stimulated high levels of receptivity when the animals had their adrenal glands, the mean lordosis quotient was significantly less than the L/M scores for the E+PR conditions following adrenalectomy ($p < .05$).

Correlated with the decreased receptivity scores was severe lethargy when the adrenalectomized animals were

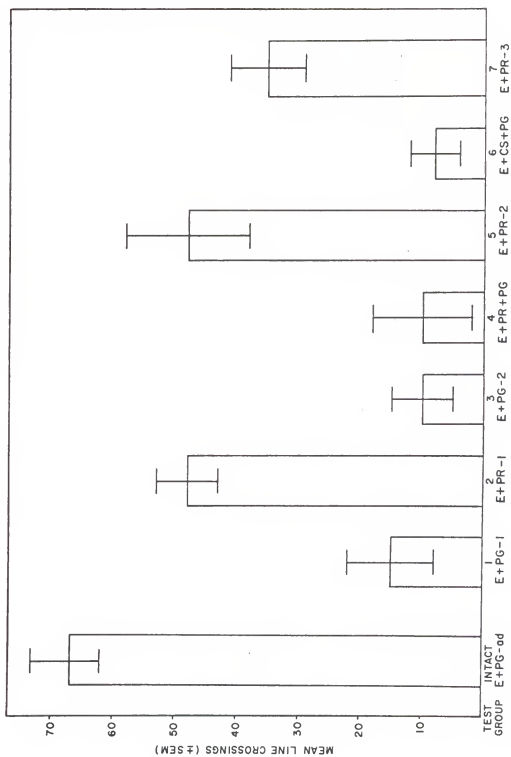
FIGURE 5. Receptivity scores of ovariectomized and adrenalectomized rats tested at 7 weekly intervals with sc estrogen and PGE_2 , progesterone and/or corticosterone. See text and Table 11 for explanation of the codes.



implanted with PGE₂ (Figure 6). A single-factor analysis of variance for repeated measures followed by a Newman-Keuls a posteriori test revealed that the (E+PG-ad) paradigm resulted in more activity than the (E+PR-1) and (E+PR-2) treatments carried out following adrenalectomy ($p < .05$), although all of these treatments resulted in moderate to high levels of activity. But PGE₂ resulted in significantly reduced and low levels of activity when implanted into the animals following adrenalectomy ($p < .01$). Systemic injections of progesterone (E+PR+PG) or corticosterone (E+CS+PG) were unable to overcome the PGE₂ inhibition of both receptivity and activity scores.

With the exception of the lethargy following PGE₂ implants, the animals appeared in good health following all of the treatments. When the animals were tested with systemic E₂B and progesterone during and at the conclusion of the experiment, high levels of receptivity and activity were observed.

FIGURE 6. Open field activity scores of ovariectomized and adrenalectomized rats tested at 7 weekly intervals with sc estrogen and PGE₂, progesterone and/or corticosterone. See text and Table 11 for explanation of the codes.



DISCUSSION

Experiments 1 through 4 have revealed that PGE_2 is able to stimulate sexual receptivity in ovariectomized, estrogen primed, female rats. When the intraventricular route of administration was employed, low but significant levels of receptivity were observed. One explanation for the low lordotic scores could have been the rapid catabolism of the PGE_2 following this route of administration. Low levels of prostaglandin degradative enzymes have been demonstrated in the brain (Nakano, Prancan and Moore, 1972) and there is considerable evidence that catabolism of brain prostaglandins occurs outside of the central nervous system. Since uptake of radiolabelled prostaglandin is relatively high in the choroid plexus (Bito, 1972a,b) it has been suggested that prostaglandins synthesized in the brain are absorbed into the venous circulation via CSF and degraded primarily in other tissues such as the lung and liver (Wolfe, 1975). Therefore, the low levels of receptivity following intraventricular implants of PGE_2 could have been due to the rapid absorption of the compound by the choroid plexus and passage into the systemic venous circulation. Another explanation for the low receptivity scores is that diffusion of the PGE_2

into the periventricular space and surrounding neuropil resulted in very little of the compound reaching specific loci mediating sexual receptivity. Although the implants were placed in the third ventricle in the region of the anterior hypothalamus and POA, diffusion throughout the ventricular system could have resulted in the PGE_2 exerting an effect at a variety of brain regions. To determine if the behavioral effects of PGE_2 were due to the compound's action in specific areas of the brain a separate group of animals was tested following implants of PGE_2 into three discrete brain regions.

In Experiment 2, unilateral implants of PGE_2 were directed into the region of preoptic area (POA)-anterior hypothalamus, medial basal hypothalamus (MBH) and Interpeduncular nucleus (IP). For the subgroup receiving implants within the neuropil, PGE_2 stimulated lordosis quotients greater than 50 only when implanted in the POA-anterior hypothalamic region. Receptivity was not observed in the group with cannulas extending out of the brain in the POA-anterior hypothalamic region. Because of the evidence discussed in the Introduction that LRF is probably located within, and controlled partially by the neurons in this same general brain region (Zimmerman, 1976), these data support the hypothesis that PGE_2 exerts its effects via a LRF mechanism. Especially since implants of PGE_2 into the POA and MBH have been found to increase plasma LH titers (Ojeda, Jameson and McCann, 1976). It was observed that

PGE₂ stimulated low levels of receptivity in the MBH and IP. Although the scores were less than 50 the L/M levels were significantly higher than the control scores in these same regions. Perikarya containing LRF are thought to be located in the arcuate nucleus within the MBH region (Barry et al., 1974). As in the case of the POA implants, an LRF mechanism could explain the moderate lordosis quotient observed with PGE₂ in the MBH. It would be rather speculative, however, to suggest that such an LRF mechanism was the cause of the slight lordosis quotient observed with PGE₂ in the IP. One would have to propose a model involving PGE₂ and an intermediate neurotransmitter since LRF activity has not been localized in this region of the mesencephalon. Such a mechanism is highly unlikely in view of the evidence that PGE₂ stimulated LH release is not affected by a variety of neurotransmitter blockers (Harms, Ojeda and McCann, 1976). A more parsimonious interpretation of the data is that the anterior hypothalamus is the region of the brain most sensitive to the behavioral effects of PGE₂ and that the minimal effects observed in the MBH and IP were due to leakage into the POA. This interpretation is also unlikely, though, since implants at the base of the brain in the anterior hypothalamus were closer to the POA than the implants in the IP, yet they induced even lower L/M scores. Another argument against leakage is that the well established hyperthermic response induced by PGE₂ was equally intense following implants in

both the POA and MBH. But no significant rise in body temperature was observed when PGE_2 was implanted into the IP region.

Although not significant, when compared with control values, a mean lordosis quotient of 68 was observed in those animals with PGE_2 tapped canullas that extended out of the brain in the region of the MBH. The mechanism by which the PGE_2 was able to stimulate receptivity in this area could well have been via ACTH stimulated adrenal progesterone. Vale, Rivier and Guillemin (1971) found increases in ACTH secretion from incubated pituitaries following prostaglandin E_1 and Hedge and Henson (1972) reported in vivo ACTH release following the injection of 0.5 to 1 μg of prostaglandin into the basal hypothalamus. A similar release of ACTH could have occurred in those animals with PGE_2 implanted in the sub-arachnoid space ventral to the MBH. This increased release of ACTH could have resulted in a subsequent release of adrenal progesterone which in turn stimulated sexual receptivity.

The implant data discussed thus far are from those animals that met the a priori criteria of not responding to E_2B alone and showing L/M scores of 50 or higher on the E_2B and progesterone test either preceding or following a given implant test. These animals also had to meet an a posteriori criteria of not responding to the cholesterol implants in order to be included in the data analysis. Since the latter criteria was established a posteriori, Table 5 also

includes the results of analyzing the data from animals failing to meet this criteria. This analysis revealed a confounding factor due to the procedure of implanting the cannulas. The L/M scores under the control conditions followed the same trend that was apparent following PGE_2 implants in the three regions studied. Although in the POA and MBH, PGE_2 induced L/M scores that were significantly greater than the control values, the scores for the MBH control condition were significantly higher than the IP controls. Thus, part of the facilitatory effect of implanting PGE_2 was due to the implant procedure and trauma. Trauma has been shown to increase prostaglandin levels (Wolfe, 1975), and the present results have shown that PGE_2 can induce receptivity in estrogen primed rats. Therefore, the facilitation observed under the control conditions could well have been due to PGE_2 released endogenously.

Stimulation of adrenal progesterone has also been found to result in sexual receptivity in estrogen primed, ovariectomized female rats and could have elicited the behavior observed in the present investigation. If this was the mechanism underlying the prostaglandin effects, then adrenalectomy should have abolished the behavioral effects of PGE_2 . In Experiment 4, adrenalectomy did block PGE_2 induced receptivity. However, the same experiment revealed that this block was not due solely to the elimination of adrenal progesterone. Activity levels were also significantly depressed. Adrenalectomy by itself did not

result in appreciable decreases in activity. Nor did implantation of PGE_2 into the anterior hypothalamus of animals with their adrenals intact. But the combination of adrenalectomy and PGE_2 implants led to a significant decrease in open field activity levels. This inhibition was sufficient to block receptivity even when the animals were primed with systemic E_2B and progesterone. Without the PGE_2 implant, E_2B and progesterone elicited lordosis quotients that were even greater than scores elicited following this paradigm in animals with their adrenals. Lethargy has been reported as a behavioral consequence of PGE_2 administration, although in the present series of experiments, unilateral PGE_2 implants caused no change in activity levels. Consequently, it is hypothesized that the combination of PGE_2 administration and adrenalectomy led to a synergistic effect and that severe lethargy was the result. That the adrenalectomy block of PGE_2 induced receptivity was due to decreased activity levels and not to the absence of adrenal progesterone was also suggested by the results of Experiment 1. In that study, all of the animals were pretreated with a dose of dexamethasone known to inhibit ACTH release and subsequent adrenal progesterone (Paris et al., 1971). In spite of the dexamethasone treatment, L/M scores were significantly elevated over control values.

Evidence from Experiment 1 suggests that the PGE_2 effects are transient. At 165 minutes, the mean lordosis

quotient was 65 for the PGE₂ implanted group which was significantly higher than the score of 18 for the cholesterol animals at this time point. Some animals had shown their maximum scores as early as 45 to 75 minutes after implantation. It is also important that all but one of the animals were showing diminished scores at the time of the final test. Thus, the PGE₂ induced receptivity was a transient phenomena that showed a decline by 240 minutes. At 210 minutes, body temperature was also no longer elevated over control values in the PGE₂ treated animals. This finding suggests that the potency of the PGE₂ to induce hyperthermia was related to the ability of the compound to induce receptivity.

The failure of indomethacin to block receptivity in estrogen and progesterone primed rats is not easily interpreted. Possible explanations are that indomethacin does not block prostaglandin synthesis in the brain or that prostaglandin synthesis is not part of the mechanism by which estrogen and progesterone induce receptivity. As discussed in the Introduction the site of action of indomethacin blockage of ovulation is still controversial. Experiment 3, however, was a very close replication of a procedure used by Ojeda et al. (1975) in which indomethacin was found to inhibit LH release when implanted in the MBH or injected systemically. In Experiment 3, systemic injections of comparable indomethacin doses failed to inhibit receptivity. Close inspection of the data in Experiment 3

does suggest that at the 5 and 7.5 mg doses, a slight, but nonsignificant decrease in receptivity did result. The 15 mg dose did inhibit receptivity, but since this high dose also caused marked deterioration of the animals' health, the behavioral inhibition cannot be attributed solely to inhibition of prostaglandin synthesis. Especially since activity levels were diminished to the same extent that receptivity was inhibited under all three conditions of systemic indomethacin administration.

When the indomethacin was implanted directly into the anterior hypothalamus, there was no observable change in the animals' health, normal levels of activity were observed, and if anything, a potentiation of sexual receptivity resulted. Using 30 gauge cannulas, Ojeda et al. (1975) were able to block LH release following hypothalamic implants of diluted indomethacin. Intravenous LRF overcame this block. This evidence would suggest that indomethacin is able to act at the level of the hypothalamus following intracerebral administration. Because indomethacin failed to inhibit receptivity in estrogen and progesterone primed animals in Experiment 3, it is possible that neither prostaglandins nor LRF are part of the mechanism by which estrogen and progesterone exert their effects on sexual receptivity.

The finding that bilateral implants of PGE_2 into the anterior hypothalamus induced severe lethargy and rapid death due to hemorrhage was not expected. Although small

hemorrhages were observed in two animals used in Experiment 1 in which unilateral implants were employed, this vascular reaction was attributed to the combined effects of PGE_2 and dexamethasone. PGE_2 has been implicated in causing vasospasm (Kapp, Mahaley and Odom, 1968) and dexamethasone can lead to increased blood pressure (Kjellstrand, 1975). The simultaneous occurrence of these two conditions could be expected to result in a ruptured vessel. Dexamethasone was not used in Experiment 2 and none of the animals was found to have suffered from hemorrhage. Not until the dose of PGE_2 was effectively doubled by using bilateral cannulas was the condition again observed. Sixty percent of the animals treated using the bilateral implants were found to have suffered from massive intracerebral hemorrhage. Because the animals in the present study had all been primed with subcutaneous E_2B , it is possible that an interaction occurred between the estrogen and PGE_2 . Estrogen can block platelet aggregation and has been shown to stimulate synthesis of PGE_2 in platelets (Mitchell, 1974; Goldberg, Maish, Ramwell and Ramey, 1976). Thus it does not seem unreasonable to propose that estrogen and PGE_2 might interact in the brain to cause hemorrhage. Interestingly, the incidence of both ischemic and hemorrhagic stroke increases in women

during pregnancy (Cross, Castro and Jennett, 1968) when estrogen levels are elevated. Estrogen levels were also high in the animals suffering hemorrhagic stroke in the present study.

Conclusion

Although the present series of experiments has revealed a facilitatory effect of PGE_2 upon lordotic behavior in female rats, the data do not confirm a mechanism by which this effect is exerted. Adrenalectomy in Experiment 4 resulted in the abolition of PGE_2 's facilitatory effect. Therefore, one could conclude that the effects were due to PGE_2 stimulated ACTH and adrenal progesterone release. However, the adrenalectomized animals were also extremely lethargic as measured in the open-field activity chamber. This lethargy was sufficient to prevent receptivity following sc E_2B and progesterone and could not be overcome with corticosterone therapy. Therefore, Experiment 4 failed to define the role that adrenal progesterone might play in PGE_2 induced sexual receptivity.

Evidence that the facilitation of lordotic behavior following PGE_2 was due to some mechanism other than adrenal progesterone release was obtained during the course of Experiment 1. All of the animals receiving intraventricular PGE_2 in that first study were treated with 250 $\mu\text{g}/\text{kg}$ of

dexamethasone. This dose of the synthetic corticosteroid has been shown to inhibit ACTH release and subsequent stimulation of adrenal progesterone (Paris et al., 1971). No lethargy was apparent in the animals treated with the dexamethasone suggesting that the decreased activity observed in Experiment 4 was due to adrenal hormones other than those controlled by ACTH. This conclusion is consistent with the finding in Experiment 4 that the lethargy could not be reversed with sc progesterone or corticosterone. Additional evidence that the PGE₂ induced receptivity was not due solely to adrenal progesterone is to be found in the studies of others. Moss et al. (personal communication) treated ovariectomized, estrone primed rats with systemic PGE₂ and observed lordosis quotients indicative of receptivity in both intact and adrenalectomized animals.

Although the Moss et al. results and those of Experiment 1 do not support the hypothesis that adrenal progesterone release was paramount for the display of receptivity following PGE₂, the results also do not preclude this sequence as an alternative mechanism under certain conditions. Since ACTH release and stimulation of adrenal progesterone are known to occur following the administration of PGE₂ in the MBH (Hedge, 1976), it could very well account for the lordotic responding observed following implants of PGE₂ into the sub-arachnoid space ventral to the MBH in Experiment 2.

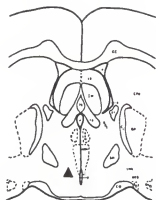
The evidence from Experiment 3 suggests that the

mechanism by which PGE_2 exerts its effects is not part of the mechanism by which estrogen and progesterone facilitate sexual receptivity in female rats. Since the paradigm used during the indomethacin experiment should have inhibited LRF as well as prostaglandin synthesis (Ojeda, Wheaton and McCann, 1975) the results suggest that LRF stimulation is also not part of the mechanism by which the gonadal steroids stimulate female receptivity. This conclusion does not preclude the possibility that stimulation of brain PGE_2 and LRF is not part of the sequence when receptivity is induced using chronic estrogen instead of estrogen plus progesterone. Evidence suggesting that the two paradigms do not act via the same mechanism was described in the Introduction. Until some of the alternative interpretations can be put to the experimental test, the precise role that PGE_2 might play in facilitating female sexual receptivity will remain a mystery. However, the present data suggest that such a role is probable and worthy of further exploration.

APPENDIX

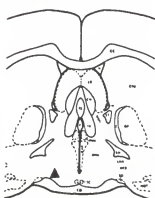
APPENDIX FIGURE 1. Sites of PGE₂ implants in animals used in Experiment 2. Numbers to the left of the diagrams represent the individual animal's number and correspond to those used in Tables 5 through 8. Only those cannula sites that were positively identified from histology are represented. Triangles with the apex pointing up indicate a cannula tract that ended within the neuropil. Inverted triangles indicate tracts that extended into the sub-arachnoid space. The number in the lower left corner of each brain section illustrated represents the number of mm anterior to stereotaxic zero. The numbers in the lower right corner represent the receptivity quotients following PGE₂ and cholesterol respectively. NT indicates animal was not tested under the designated condition.

1

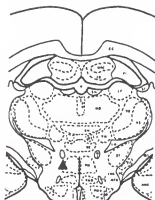


6.8 100 : 90

2



7.0 80 : 40

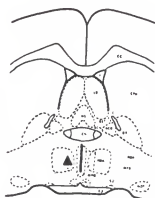


4.8 80 : 0

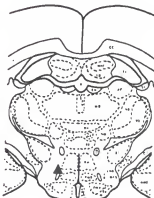


1.6 NT : NT

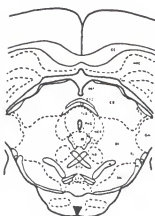
3



7.4 90 : 30



4.8 80 : 40



1.8 30 : 20

4

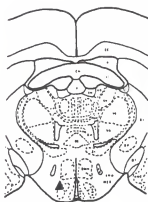


7.4 40 : 10



2.2 0 : 0

6



5.4 0 : 0

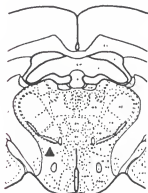


0.8 50 : 0

7



7.2 20 : 100

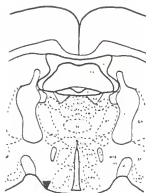


5.2 0 : 40

8



7.8 100 : 20

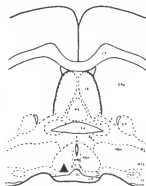


6.0 80 : 0



3.4 20 : 0

9



7.6 60 : 50



5.6 0 : 10

10

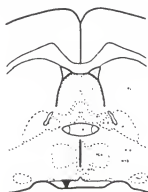


7.8 30 : 11



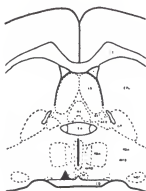
5.8 NT : NT

11

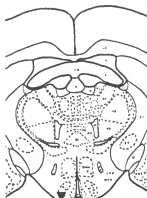


7,4 0 : 0

13

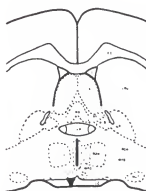


7,4 100 : 100

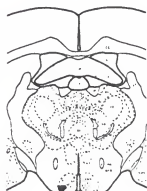


5,4 80 : 0

14



7,4 100 : 70



5,6 60 : 90



1,6 70 : 30

16



5.0 100 : 20



1.8 0 : 0

17



7.4 0 : 0



5.0 20 : 0



1.8 30 : 0

18



7.4 90 : 10



5.0 NT : NT



2.0 NT : 0

19

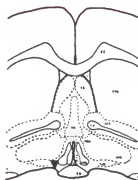


5.2 20 : 40

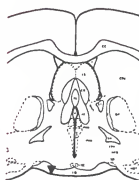


2.4 0 : 11

20



8.0 90 : 117



7.0 90 : 90



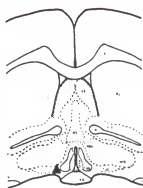
2.2 10 : 0

21



2.0 0 : 0

23



8.0 90 : 70

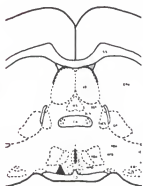


5.2 0 : NT

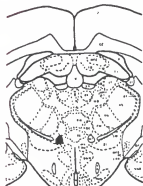


2.2 0 : 0

25



7.2 90 : 0

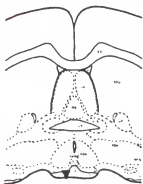


5.0 90 : 40



1.6 10 : 0

26



7.6 0 : 30



5.4 100 : 70

28



7.8 70 : 40

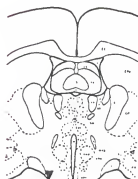


5.8 NT : 30



2.0 80 : 0

29



6.4 20 : 0



2.0 NT : 20

30



7.8 90 : 0



5.6 NT : NT



7.0 80 : 70

REFERENCES

- Ahlenius, S., J. Engel, H. Eriksson, and P. Sodersten. 1972. Effects of tetrabenzazine on lordosis behavior and on brain monoamines in the female rat. J. Neural Trans. 33:155.
- Ahlenius, S., J. Engel, H. Eriksson, K. Modish, and P. Sodersten. 1972. Importance of central catecholamines in the mediation of lordosis behavior in ovariectomized rats treated with estrogen and inhibitors of monoamine synthesis. J. Neural Trans. 33:247.
- Armstrong, D. T. and D. L. Grinwich. 1972. Blockade of spontaneous and LH-induced ovulation in rats by indomethacin, an inhibitor of prostaglandin biosynthesis. Prostaglandins 1:21.
- Barracrough, C. A. and B. A. Cross. 1963. Unit activity in the hypothalamus of the cyclic female rat: effect of genital stimulation and progesterone. J. Endocr. 26:339.
- Barry, J., M. P. Dubois, and B. Carett. 1974. Immunofluorescence study of the preoptico-infundibular LRF neurosecretory pathway in the normal, castrated or testosterone-treated male guinea pig. Endocrinology 95:1416.
- Barry, J., M. P. Dubois, and P. Poulain. 1973. LRF producing cells of the mammalian hypothalamus. Z. Zellforsch. 146:351.
- Batta, S. K., M. Zanisi, and L. Martini. 1974. Prostaglandins and gonadotropin secretion. Neuroendocrinology 14:224.
- Behrman, H. R., G. P. Orczyk, and R. O. Greep. 1972. Effect of synthetic gonadotrophin-releasing hormone (GH-RH) on ovulation blockade by aspirin and indomethacin. Prostaglandins 1:245.
- Bito, L. Z. 1972a. Comparative study of concentrative prostaglandin accumulation by various tissues of mammals and marine vertebrates and invertebrates. Comp. Biochem. Physiol. 43A:65.
- Bito, L. Z. 1972b. Accumulation and apparent active transport of prostaglandins by some rabbit tissues in vitro. J. Physiol. 221:371.

- Bjorklund, A., O. Lindvall, and A. Nobin. 1975. Evidence of an incerto-hypothalamic dopamine neurone system in the rat. Brain Research 89:29.
- Blondaux, C., A. Juge, F. Sordet, G. Chouvet, M. Jouvét, and J. F. Pujol. 1973. Modification du métabolisme de la sérotonine (5-HT) cérébrale induite chez le rat par administration de 6-hydroxy-dopamine. Brain Research 50:101.
- Brody, M. J. and P. J. Kadowitz. 1974. Prostaglandins as modulators of the autonomic nervous system. Fed. Proc. 33:48.
- Carey, R. J. 1972. A neuroanatomical investigation of enhanced cutaneous gustatory responsivity associated with septal forebrain injury. J. Comp. Physiol. Psychol. 80:449.
- Carrer, H., G. Asch, and C. Aron. 1973/1974. New facts concerning the role played by the ventromedial nucleus in the control of estrous cycle duration and sexual receptivity in the rat. Neuroendocrinology 13:129.
- Castracane, V. D. and S. K. Saksena. 1974. Prostaglandins of the E series and LH release in fertile male rats. Prostaglandins 7:53.
- Chobsieng, P., Z. Naor, Y. Koch, U. Zor, and H. R. Lindner. 1975. Stimulatory effect of prostaglandin E₂ on LH release in the rat: evidence for hypothalamic site of action. Neuroendocrinology 17:12.
- Clark, G. 1942. Sexual behavior in rats with lesions in the anterior hypothalamus. American J. Physiol. 137:746.
- Collins, W. P., J. A. Elias, J. F. Henman, and D. A. Johnson. 1974. The role of prostaglandins in human reproductive function. Acta European Fertil. 5:119.
- Conrad, L. C. A., C. M. Leonard, and D. W. Pfaff. 1974. Connections of the medial and dorsal raphe nuclei in the rat: an autoradiographic and degeneration study. J. Comp. Neurol. 156:179.
- Conrad, L. A. and D. W. Pfaff. 1974. Autoradiographic tracing of projections from preoptic area and anterior hypothalamus in the rat. Soc. Neuroscience 4th Ann. Meeting, Abstract 136.
- Cragg, B. J. 1961. The connections of the habenular in the rabbit. Exptl. Neurol. 3:388.

- Cross, J. N., P. O. Castro, and W. B. Jennet. 1968. Cerebral strokes associated with pregnancy and the puerperium. British Med. J. 3:214.
- Czaja, J. A., D. A. Goldfoot, and H. J. Karavolas. 1974. Comparative facilitation and inhibition of lordosis in the guinea pig with progesterone, 5 α -pregnane-3,20-dione, or 3 α -hydroxy-5 α -pregnan-20-one. Horm. Behav. 5:261.
- Dembinska, A., L. Grodzinska and J. Piotrowicz. 1974. The influence of indomethacin on the prostaglandins synthetase system in rat tissues in vivo. Polish J. Pharmacol. Pharm. 26:79.
- De Wied, D. and W. de Jong. 1974. Drug effects and hypothalamic-anterior pituitary function. Ann. Rev. Pharmacol. 14:389.
- Donoso, A. O. and J. O. Cukier. 1968. Oestrogen as a depressor or noradrenaline concentration in the anterior hypothalamus. Nature 218:969.
- Donoso, A. O. and F. J. E. Stefano. 1967. Sex hormones and concentration of noradrenaline and dopamine in the anterior hypothalamus of castrated rats. Experientia 23:665.
- Eriksson, H. and P. Sodersten. 1973. A failure to facilitate lordosis behavior in adrenalectomized and gonadectomized estrogen-primed rats with monoamine synthesis inhibitors. Horm. Behav. 4:89.
- Eskay, R. L., J. Warberg, R. S. Mical, and J. C. Porter. 1975. Prostaglandin E₂-induced release of LHRH into hypophyseal portal blood. Endocrinology 97:816.
- Espino, C., M. Sano, and G. N. Wade. 1975. Alpha-methyltryptamine blocks facilitation of lordosis by progesterone in spayed, estrogen-primed rats. Pharmacol. Biochem. Behav. 3:557.
- Everitt, B. J., K. Fuxe, and T. Hokfelt. 1974. Inhibitory role of dopamine and 5-dyfroxytryptamine in the sexual behaviour of female rats. European J. Pharmacol. 29:187.
- Ferreira, S. H. and J. R. Vane. 1974. New aspects of the mode of action of nonsteroid anti-inflammatory drugs. Ann. Rev. Pharmacol. 14:57.
- Gilmore, D. P. and A. A. Shaikh. 1972. The effect of prostaglandin E₂ in inducing sedation in the rat. Prostaglandins 2:143.

- Goldberg, V., T. Maish, P. W. Ramwell, and E. Ramey. 1976. Effects of gonadal steroids on prostaglandin synthesis in platelets. Fed. Proc. 35:779.
- Gorzalka, B. B. and R. E. Whalen. 1974. Inhibition, not facilitation of sexual behavior by PCPA. Pharmacol. Biochem. Behav. 3:511.
- Gumalka, W., R. Samanin, L. Valzelli, and S. Consolo. 1971. Behavioural and biochemical effects following the stimulation of the nucleus raphis dorsalis in rats. J. Neurochem. 18:533.
- Halasz, B. 1969. The endocrine effect of isolation of the hypothalamus from the rest of the brain. In: Frontiers in Neuroendocrinology, 1969, (Ganong, W. F. and L. Martini, Eds.) Oxford Univ. Press, New York, p. 307.
- Hall, N. R. and W. G. Luttge. 1974. Intracerebral progesterone: effects on sexual behavior in female mice. Soc. Neuroscience 4th Ann. Meeting, Abstract 270.
- Hardy, D. F. and D. M. Warburton. 1969. Cholinesterase inhibition and sexual receptivity in the female rat. Psychonomic Sci. 17:311.
- Harms, P. G., S. R. Ojeda, and S. M. McCann. 1973. Prostaglandin involvement in hypothalamic control of gonadotropin and prolactin release. Science 181:760.
- Harms, P. G., S. R. Ojeda, and S. M. McCann. 1974. Prostaglandin-induced release of pituitary gonadotropins: central nervous and pituitary sites of action. Endocrinology 94:1459.
- Harms, P. G., S. R. Ojeda, and S. M. McCann. 1976. Failure of monoaminergic and cholinergic receptor blockers to prevent prostaglandin E₂-induced luteinizing hormone release. Endocrinology 98:318.
- Hedge, G. A. 1976. Hypothalamic and pituitary effects of prostaglandins on ACTH secretion. Prostaglandins 11:293.
- Hedge, G. A. and S. D. Henson. 1972. The effects of prostaglandins on ACTH secretion. Endocrinology 91:925.
- Herndon, J. G. and D. B. Neill. 1973. Amphetamine reversal of sexual impairment following anterior hypothalamic lesions in female rats. Pharmacol. Biochem. Behav. 1:285.

- Ho, G. K. W., D. M. Quadagno, P. H. Cooke, and R. A. Gorski. 1973/1974. Intracranial implants of actinomycin-D: effects on sexual behavior and nucleolar ultrastructure in the rat. Neuroendocrinology 13:47.
- Hough, J. C., G. K. W. Ho, P. H. Cooke, and D. M. Quadagno. 1974. Actinomycin-D: reversible inhibition of lordosis behavior and correlated changes in nucleolar morphology. Horm. Behav. 5:367.
- Hinman, J. W. 1972. Prostaglandins. Ann. Rev. Biochem. 41:161.
- Janowski, D. S. and J. M. Davis. 1970. Progesterone-estrogen effects on uptake and release of norepinephrine by synaptosomes. Life Sciences 9:535.
- Jouvet, M. 1972. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. Ergebn. Physiol. 64:166.
- Kalra, S. P. and S. M. McCann. 1973. Effect of drugs modifying catecholamine synthesis on LH release from preoptic stimulation in the rat. Endocrinology 93:356.
- Kapp, J., M. S. Mahaley, and G. L. Odom. 1968. Cerebral arterial spasm: experimental evaluation of mechanical and humoral factors in pathogenesis. J. Neurosurgery 29:350.
- Kizer, J. S., M. Palkovits, and M. J. Brownstein. 1976. Releasing factors in the circumventricular organs of the rat brain. Endocrinology 98:311.
- Kjellstrand, C. M. 1975. Side effects of steroids and their treatment. Transplantation Proceedings 7:123.
- Komisaruk, B. R. 1974. Neural and hormonal interactions in the reproductive behavior of female rats. In: Reproductive Behavior, (Montagne, W. and W. A. Sadler, Eds.) Plenum Press, New York, p. 97.
- Komisaruk, B. R., K. Larsson, and R. Cooper. 1972. Intense lordosis in the absence of ovarian hormones after septal ablation in rats. Soc. Neuroscience 2nd Ann. Meeting, Abstract 51.10.
- Komisaruk, B. R., P. G. McDonald, D. I. Whitmoyer, and C. H. Sawyer. 1967. Effects of progesterone and sensory stimulation on EEG and neuronal activity in the rat. Exptl. Neurol. 19:494.

- Kordon, C., B. Kerdelhue, E. Pattou, and M. Jutisz. 1974. Immunocytochemical localization of LHRH in axons and nerve terminals of the rat median eminence. Proc. Soc. Exptl. Biol. Med. 147:122.
- Kostowski, W., R. Samanin, S. R. Bareggi, V. Marc, S. Garattini, and L. Valzelli. 1974. Biochemical aspects of the interaction between midbrain raphe and locus coeruleus in the rat. Brain Research 82:178.
- Kostowski, W., E. Giacalone, S. Garattini, and L. Valzelli. 1969. Electrical stimulation of midbrain raphe: biochemical, behavioral and bioelectrical effects. European J. Pharmacol. 7:170.
- Kow, L. M., C. W. Malsbury and D. W. Pfaff. 1974. Effects of progesterone on female reproductive behavior in rats: possible modes of action and role in behavioral sex differences. In: Reproductive Behavior, (Montagna, W. and W. A. Sadler, Eds.) Plenum Press, New York, p. 179.
- Lau, I. F. and S. K. Saksena. 1974. Prostaglandin E₂ and LH release in ovariectomized rats. Prostaglandins 7:49.
- Libertun, C. and S. M. McCann. 1973. Blockade of the release of gonadotropins and prolactin by subcutaneous or intraventricular injection of atropine in male and female rats. Endocrinology 92:1714.
- Liebeskind, J. C., G. Guilbaud, J. Bessen, and J. L. Oliveras. 1973. Analgesia from electrical stimulation of the periaqueductal gray matter in the cat: behavioral observations and inhibitory effects on spinal cord interneurons. Brain Research 50:441.
- Lindstrom, L. and B. J. Meyerson. 1966. Effects of cholinergic and anticholinergic agents on estrogen-progesterone activated estrus behavior in ovariectomized rats. Acta Physiol. Scandinavia Suppl. 277:121.
- Lindstrom, L. and B. J. Meyerson. 1970. The effect of atropine on oestrous behaviour in female rats after increased or decreased central nervous monoamine levels. Acta Pharmacol. Suppl. 1:65.
- Lindvall, O. and A. Bjorklund. 1975. The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. Acta Physiol. Scandinavia Suppl. 412:1.

- Lints, C. E. and J. A. Harvey. 1969. Altered sensitivity to footshock and decreased brain content of serotonin following brain lesions in the rat. J. Comp. Physiol. Psychol. 67:23.
- Lisk, R. D. 1962. Diencephalic placement of estradiol and sexual receptivity in the female rat. American J. Physiol. 203:493.
- Luttge, W. G. 1976. Intracerebral implantation of progesterone: re-examination of the brain sites responsible for facilitation of sexual receptivity in estrogen-primed ovariectomized rats. Physiol. Behav. (in press).
- Luttge, W. G. and N. R. Hall. 1976. Interactions of progesterone and dihydroprogesterone with dihydrotestosterone on estrogen activated sexual receptivity in female mice. Horm. Behav. (in press).
- Luttge, W. G. and C. J. Wallis. 1973. In vitro accumulation and saturation of ^3H -progestins in selected brain regions and in the adenohypophysis, uterus and pineal of the female rat. Steroids 22:493.
- Luttge, W. G. and R. E. Whalen. 1972. The accumulation, retention and interaction of oestradiol and oestrone in central neural and peripheral tissues of gonadectomized female rats. J. Endocr. 52:379.
- Luttge, W. G., R. B. Chronister, and N. R. Hall. 1973. Accumulation of ^3H -progestins in limbic, diencephalic, and mesencephalic regions of mouse brain. Life Sciences 12:419.
- Luttge, W. G., C. J. Wallis, and N. R. Hall. 1974. Effects of pre- and post-treatment with unlabelled steroids on the in vivo uptake of ^3H -progestins in selected brain regions, uterus, and plasma of the female mouse. Brain Research 71:105.
- Makino, T. 1973. Study on the intracellular mechanism of LH release in the anterior pituitary. American J. Obstet. Gynecol. 115:606.
- Malsbury, C. and D. W. Pfaff. 1973. Neural and hormonal determinants of mating behavior in adult male rats. In: The Limbic and Autonomic Nervous Systems: Advances in Research, (Dicara, L., Ed.) Plenum Press, New York, p. 86.
- Markee, J. E., J. W. Everitt, and C. H. Sawyer, 1952. The relationship of the nervous system to the release of gonadotrophin and the regulation of the sex cycle. Rec. Prog. Horm. Res. 7:139.

- McCann, S. M. 1971. Mechanism of action of hypothalamic-hypophysial stimulating and inhibiting hormones. In: Frontiers in Neuroendocrinology, (Martini, L. and W. F. Ganong, Eds.) Oxford Univ. Press, London, p. 209.
- McCann, S. M. 1974. Neural control of gonadotropin secretion. In: Reproductive Behavior, (Montagna, W. and W. Sadler, Eds.) Plenum Press, New York, p. 1.
- Meyerson, B. J. 1964a. Estrus behaviour in spayed rats after estrogen or progesterone treatment in combination with reserpine or tetrabenazine. Psychopharmacologia 6:20.
- Meyerson, B. J. 1964b. Central nervous monoamines and hormone induced estrus behaviour in the spayed rat. Acta Physiol. Scandinavia Suppl. 248:5.
- Meyerson, B. J. 1966. The effect of imipramine and related antidepressive drugs on estrous behaviour in ovariectomized rats activated by progesterone, reserpine or tetrabenazine in combination with estrogen. Acta Physiol. Scandinavia 67:411.
- Meyerson, B. J. 1968. Amphetamine and 5-hydroxytryptamine inhibition of copulatory behavior in the female rat. Annales Medicinæ Experimentales et Biologiae Fennae 46:394.
- Meyerson, B. J. 1973. Mechanisms of action of sex steroids on behavior; inhibition of estrogen-activated behavior by ethamoxy-triphetol (MER-25), colchicine and cyclohexamide. Prog. Brain Research 39:135.
- Mitchell, H. C. 1974. Effect of estrogens and a progestogen on platelet adhesiveness and aggregation in rabbits. J. Lab. Clin. Med. 83:79.
- Morin, L. P. and H. H. Feder. 1974. Inhibition of lordosis behavior in ovariectomized guinea pigs by mesencephalic implants of progesterone. Brain Research 70:71.
- Moss, R. L. 1974. Relationship between the central regulation of gonadotropins and mating behavior in female rats. In: Reproductive Behavior, (Montagna, W. and W. Sadler, Eds.) Plenum Press, New York, p. 55.

- Moss, R. L. and S. M. McCann. 1973. Induction of mating behavior in rats by luteinizing hormone releasing factor. Science 181:177.
- Moss, R. L., S. M. McCann, and C. A. Dudley. 1975. In: Hormones, Homeostasis and the Brain, (Gispen, W. H., Tj. B. VanWimersma Greidanus, B. Bohus, and D. De Wied, Eds.) Elsevier, Amsterdam, p. 37.
- Nakano, J., A. V. Prancan, and S. E. Moore. 1972. Metabolism of prostaglandin E₁ in the cerebral cortex and cerebellum of the dog and rat. Brain Research 39:545.
- Nance, D. W., J. Shryne, and R. A. Gorski. 1974. Septal lesions: effects on lordosis behavior and patterns of gonadotropin release. Horm. Behav. 5:73.
- Napoli, A., J. B. Powers, and E. S. Valenstein. 1972. Hormonal induction of behavioral estrus modified by electrical stimulation of the hypothalamus. Physiol. Behav. 9:115.
- Ojeda, S. R., P. G. Harms, and S. M. McCann. 1975a. Effect of inhibitors of prostaglandin synthesis on gonadotropin release in the rat. Endocrinology 97:843.
- Ojeda, S. R., H. E. Jameson, S. M. McCann. 1976. Hypothalamic area involved in prostaglandin E₂ (PGE₂)-induced LH release. Endocrine Society 58th Ann. Meeting, Abstract 270.
- Ojeda, S. R., J. E. Wheaton, and S. M. McCann. 1975b. Prostaglandin E₂-induced release of luteinizing hormone-releasing factor (LRF). Neuroendocrinology 17:283.
- O'Malley, B. W. and A. R. Means. 1974. Female steroid hormones and target cell nuclei. Science 183:610.
- Orczyk, G. P. and H. R. Behrman. 1972. Ovulation blockade by aspirin or indomethacin: in vivo evidence for a role of prostaglandin in gonadotropin secretion. Prostaglandins 1:3.
- Palkovits, M., M. Brownstein, J. M. Saavedra, and J. Axelrod. 1974. Norepinephrine and dopamine content of hypothalamic nuclei of the rat. Brain Research 77:37.
- Paris, C. A., J. A. Resko, and R. W. Goy. 1971. A possible mechanism for the induction of lordosis by reserpine in spayed rats. Biol. Reprod. 4:23.

- Pfaff, D. W. 1973. Luteinizing hormone-releasing factor potentiates lordosis behavior in hypophysectomized ovariectomized female rats. Science 182:1148.
- Potts, W. J. and P. F. East. 1972. Effect of prostaglandin E_2 on the body temperature of conscious rats and cats. Arch. Internat. Pharmace. Therapie 197:31.
- Poulain, P. and B. Carett. 1974. Iontophoresis of prostaglandins on hypothalamic neurons. Brain Research 79:311.
- Powers, B. and E. S. Valenstein. 1972. Sexual receptivity: facilitation by medial preoptic lesions in female rats. Science 175:1003.
- Powers, B. 1972. Facilitation of lordosis in ovariectomized rats by intracerebral progesterone implants. Brain Research 48:311.
- Quadagno, D. M., J. Shryne, and R. A. Gorski. 1971. Inhibition of steroid induced sexual behavior with intrahypothalamic actinomycin-D. Horm. Behav. 2:1.
- Raffel, G., P. Clarenback, B. A. Peskar, and G. Hertting. 1976. Synthesis and release of prostaglandins by rat brain synaptosomal fractions. J. Neurochem. 26:493.
- Raisman, G. 1966. The connections of the septum. Brain 89:317.
- Ramirez, V. D., B. R. Komisaruk, D. I. Whitmoyer, and C. H. Sawyer. 1967. Effects of hormones and vaginal stimulation on the EEG and hypothalamic units in rats. American J. Physiol. 212:1376.
- Ratner, A., M. C. Wilson, L. Srivastava, and G. T. Peake. 1974. Stimulatory effects of prostaglandin E_1 on rat anterior pituitary cyclic AMP and luteinizing hormone release. Prostaglandins 5:165.
- Reid, W. D. 1970. Turnover rate of brain 5-hydroxytryptamine increased by d-amphetamine. British J. Pharmacol. 40:483.
- Roberts, J. S. and J. A. McCracken. 1975. Prostaglandin $F_{2\alpha}$ production by the brain during estrogen-induced secretion of luteinizing hormone. Science 190:896.
- Ross, J., C. Claybaugh, L. Clemens, and R. A. Gorski. 1971. Short latency induction of estrous behavior with intracerebral gonadal hormones in ovariectomized rats. Endocrinology 89:32.

- Saavedra, J. M., M. Palkovits, M. J. Brownstein, and J. Axelrod. 1974. Serotonin distribution in the nuclei of the rat hypothalamus and preoptic region. Brain Research 77:157.
- Sato, T., K. Taya, T. Jyujyo, M. Hirono, and M. Igarashi. 1974. The stimulatory effect of prostaglandins on luteinizing hormone release. American J. Obstet. Gynecol. 118:875.
- Schneider, H. P. G., D. B. Crighton, and S. M. McCann. 1969. Suprachiasmatic LH-releasing factor. Neuroendocrinology 5:271.
- Setalo, G., S. Vigh, A. V. Schally, A. Arimura, and B. Flerko. 1975. LH-RH containing neural elements in the rat hypothalamus. Endocrinology 96:135.
- Singer, J. J. 1968. The effects of atropine upon the female and male sexual behavior of female rats. Physiol. Behav. 3:377.
- Smaha, L. A. and W. W. Kaelber. 1973. Efferent fiber projections of the habenular and the interpeduncular nucleus. An experimental study in the opossum and cat. Exptl. Brain Research 16:291.
- Spies, H. G., R. L. Norman, and E. C. Campbell. 1973. Luteinizing hormone release and ovulation induced by the intraventricular infusion of prostaglandin E₁ into pentobarbital blocked rats. Prostaglandins 4:131.
- Stefano, F. J. E. and A. O. Donoso. 1967. Norepinephrine levels in the rat hypothalamus during the estrous cycle. Endocrinology 81:1405.
- Sundberg, D. K., C. P. Fawcett, P. Illner, and S. M. McCann. 1975. The effect of various prostaglandins and a prostaglandin synthetase inhibitor on rat anterior pituitary cyclic AMP levels and hormone release in vitro. Proc. Soc. Exptl. Biol. Med. 148:54.
- Swanson, L. W. and B. K. Hartman. 1974. The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine- β -hydroxylase as a marker. J. Comp. Neurol. 163:467.
- Tenen, S. S. 1967. The effects of p-chlorophenylalanine, a serotonin depletor, on the avoidance acquisition, pain sensitivity and related behaviors in the rat. Psychopharmacologia 10:204.

- Terkel, A. S., J. Shryne, and R. A. Gorski. 1973. Inhibition of estrogen facilitation of sexual behavior by the intracerebral infusion of actinomycin-D. Horm. Behav. 4:377.
- Tsafriri, A., Y. Koch, and H. R. Lindner. 1973. Ovulation rate and serum LH levels in rats treated with indomethacin or prostaglandin E₂. Prostaglandins 3:461.
- Ungerstedt, U. 1971. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scandinavica Suppl. 367:1.
- Vale, W., C. Rivier, and R. Guillemin. 1971. A prostaglandin receptor in the mechanism involved in the secretion of anterior pituitary hormones. Fed. Proc. 30:363.
- Verhulst, I. and L. R. Herrenkohl. 1975. Facilitation of female sexual behavior by the luteinizing hormone-releasing factor in the medial preoptic hypothalamus. Eastern Conf. Reprod. Behav., Abstract 9.
- Weiner, R. I., R. A. Gorski, and C. H. Sawyer. 1972. Hypothalamic catecholamines and pituitary gonadotropic function. In: Brain-Endocrine Interaction, Median Eminence: Structure and Function, (Knigge, K. M., D. E. Scott, and A. Weindl, Eds.) Karger, Basel, p. 236.
- Wellman, W. and U. Schwabe. 1973. Effects of prostaglandins E₁, E₂ and F_{2α} on cyclic AMP levels in brain in vivo. Brain Research 59:371.
- Whalen, R. E., B. B. Gorzalka, J. F. DeBold, D. M. Quadagno, G. K. Ho, and J. C. Hough. 1974. Studies of the effects of intracerebral actinomycin-D implants on estrogen-induced receptivity in rats. Horm. Behav. 5:337.
- Whalen, R. E. and W. G. Luttge. 1971a. Differential localization of progesterone uptake in the brain. Role of sex, estrogen pretreatment and adrenalectomy. Brain Research 33:147.
- Whalen, R. E. and W. G. Luttge. 1971b. Role of the adrenal in the preferential accumulation of progesterin by mesencephalic structures. Steroids 18:141.
- Wheaton, J. E., J. Krulich, and S. M. McCann. 1975. Localization of luteinizing hormone-releasing hormone (LRH) in the preoptic area and hypothalamus of the rat using radioimmunoassay. Endocrinology 97

- Wirz-Justice, A., E. Hackman, and M. Lichtsteiner. 1974. The effect of oestradiol dipropionate and progesterone on monoamine uptake in the rat brain. J. Neurochem. 22:187.
- Wolfe, L. S. 1975. Possible roles of prostaglandins in the nervous system. In: Advances in Neurochemistry, Volume 1, (Agranoff, B. W. and M. H. Aprison, Eds.) Plenum Press, New York, p. 1.
- Zemlan, F. P., I. L. Ward, W. R. Crowley, and D. L. Margules. 1973. Activation of lordotic responding in female rats by suppression of serotonergic activity. Science 197:1010.
- Zimmerman, E. A. 1976. Localization of hypothalamic hormones by immunocytochemical techniques. In: Frontiers in Neuroendocrinology, Volume 4, (Martini, L. and W. F. Ganong, Eds.) Raven Press, New York, p. 25.
- Zolovic, A. J., R. Pearse, K. W. Boehlke, and B. E. Eleftheriou. 1966. Monoamine oxidase activity in various parts of the rat brain during the estrous cycle. Science 154:649.
- Zor, U., T. Kaneko, H. P. Schneider, S. M. McCann, I. P. Lowe, G. Bloom, B. Borland, and J. B. Field. 1969. Stimulation of anterior pituitary adenyl cyclase activity and adenosine 3';5' cyclic phosphate by hypothalamic extract and prostaglandin E₁. Proc. Nat. Acad. Sci. 63:918.

BIOGRAPHICAL SKETCH

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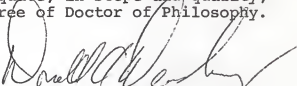
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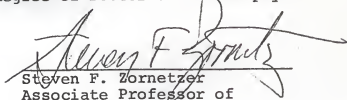
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